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(54) Title: METHOD FOR DIAGNOSIS AND TREATMENT OF RHEUMATOID ARTHRITIS

(57) Abstract: The onset and progression of chronic autoimmune diseases, including human rheumatoid arthritis (RA) are likely determined by differential expression of genes that influence inflammatory and immune responses. The collagen-induced arthritis (CIA) mouse model for RA exhibits many of the same genetic and immunological features of RA; however, the profiles of gene expression during the inflammatory and immune responses of CIA or RA have not been well characterized. Previous studies have demonstrated that mRNA levels, particularly that of cytokines, can change over the course of CIA. To determine the contribution of various genes in the pathogenesis of CIA, microarray technology was used to simultaneously monitor 8,734 target cDNAs to discover arthritic stage-specific genes. The resulting gene expression profile identified 333 genes that were at least 2-fold up-regulated in all synovial samples: normal, acute disease and chronic disease. In addition, 385 disease-specific genes were identified that were greater than or equal to 2-fold over- or under-expressed in the disease state as compared to normal synovium. Clustering analysis among the arthritic states allowed for the identification of four distinct kinetic expression patterns based on differential expression levels in normal, acute disease and chronic disease synovial samples.

METHOD FOR DIAGNOSIS AND TREATMENT OF RHEUMATOID ARTHRITIS

Government Interest in the Invention

[0001] Certain aspects of the invention disclosed herein were made with United States government support under National Institutes of Health grants AI34958, AR44059, AR47712, and AR42632. The United States government has certain rights in these aspects of the invention.

Incorporation-By-Reference Of CD-ROM Data

[0002] Applicants hereby incorporate by reference in their entirety two copies of a compact disc, labeled "Copy 1" and "Copy 2," respectively, containing table1.1.txt, 2,276,363 size in bytes, created on October 31, 2002; table1.2.txt, 1,335,492 size in bytes, created on October 31, 2002; table1.3.txt, 2,924,772 size in bytes, created on October 31, 2002; table2.1.txt, 817,381 size in bytes, created on October 31, 2002; table2.2.txt, 1,003,344 size in bytes, created on October 31, 2002; and table2.3.txt, 604,772 size in bytes, created on October 31, 2002.

Background of the Invention

Field of the Invention

[0003] The invention relates generally to materials and methods for diagnosis and treatment of rheumatoid arthritis (RA) and related conditions. More specifically, the invention relates to nucleic acids, proteins, arrays thereof, methods for diagnosis and methods for analyzing the severity of RA and related conditions using, for example, patterns of up- and down-regulation of specific genes identified by microarray technology. The invention further relates to the treatment of RA by activating those genes or proteins that are down-regulated and/or inhibiting those genes or proteins that are up-regulated. The invention also relates to identifying and using targets for drug treatment, methods of screening candidate drugs, and methods for identifying optimal treatment approaches for a specific patient.

Description of the Related Art

[0004] Collagen-induced arthritis (CIA) in mice has been utilized to study underlying mechanisms of autoimmune arthritis because of its clinical, histologic, immunologic and genetic similarity to rheumatoid arthritis (RA). Although several immunoregulatory genes have been implicated in this model system, molecular mechanisms underlying the pathophysiology have only been partially defined.

[0005] In CIA, progression of disease is associated with changes in the cell types infiltrating the joint. The acute phase of the disease is characterized by a predominantly neutrophilic infiltrate, with monocytes and lymphocytes constituting approximately 5% of the inflammatory cell population. By day 49, a decrease in lymphocytes is observed, with an increase in fibroblast/macrophage type cells and an increasingly fibrotic appearance. In conjunction with the changes of cellular infiltrate, mRNA and protein expression levels of several cytokines and chemokines also change over the course of disease. For example, TNFα protein expression in the

joint precedes that of IL-1β and IFN-γ is expressed shortly after disease onset, but not late in disease. IL-1β and IL-10 mRNAs, but not those of IFN-γ and IL-5, are detected in late disease.

[0006] Classical approaches to studying inflammatory mediators in arthritis have focused on identifying and analyzing these mediators individually. While this method has proven extremely productive, arthritis represents a complex and multifactorial pathophysiology that likely involves hundreds or thousands of individual gene products acting in concert. Improved understanding of the genes that are operative during the development of the inflammatory lesion may aid in the design of disease-specific therapies. Several methods to examine coordinated gene expression have been developed, including Northern blot, ribonuclease protection assay (RPA), differential display and sequencing of cDNA libraries and expressed sequence tags (ESTs). Using total paw RNA from a mouse with CIA and using the method of RPA, the inventors have previously demonstrated distinct changes in mRNA expression of a number of cytokines in early and late CIA. IL-2, IL-6, MIP2 and IL-1β were found predominantly in early disease, whereas, TGFβ was found predominantly in late disease. IL-11, IL-1ra, MIP1α, RANTES, TNFα and TNFβ were present both in early and late disease. These changes in gene expression within the joint likely affect the disease pathology observed at the cellular and macroscopic level. Whether a similar temporal change in cellular infiltrate and mRNA expression profiles also occurs in RA is not clear, as few synovial biopsies have been performed at the very early stages of RA. However, since most of the previously mentioned cytokines are found in synovial fluids and chronic RA synovium, these findings have relevance to RA.

[0007] The recent advent of high-throughput methods, such as serial analysis of gene expression (SAGE) and DNA microarrays, have allowed large-scale, genome-wide characterizations of gene expression to be performed. Whole-genome expression profiling represents a major advance in genome-wide functional analysis. In a single assay, the transcriptional response of each gene to a change in cellular state, including a disease or a chemical perturbation, can be measured. These changes in gene expression can reflect changes in mRNA levels or changes in the cells (proliferation or infiltration) that synthesize these mRNAs. DNA microarray technology is well-suited for analyzing chronic diseases, such as autoimmune arthritis, because of the wide spectrum of genes and endogenous mediators involved. A recent report describing the analysis of RA and inflammatory bowel disease tissues used a microarray of about 100 genes known to have a role in inflammation. IL-6 and several matrix metalloproteinases were markedly upregulated in RA tissues; however the observed upregulation of matrix metallo-elastase (HME) was unexpected, since its expression was previously thought to be limited to alveolar macrophages and placental cells. Analyses such as these are able to identify genes, both known and novel, and discover their coordinately regulated expression during the disease process.

[0008] Analysis of global gene expression in disease joints is likely to lead to a fuller understanding of the inflammatory processes responsible for arthritis. In the present study, DNA microarray technology was used to identify novel genes and biological pathways involved in CIA and to test the hypothesis that the previously observed set of stage-specific differentially activated genes in CIA represents a larger transcriptional profile.

Summary of the Invention

[0009] Using microarray analysis, the expression of 8734 cDNAs was analyzed during various stages of mouse collagen induced arthritis (CIA), an animal model of RA. From the results, a method for the diagnosis and treatment of RA was developed.

[0010] Embodiments relate to methods for the diagnosis and analysis of autoimmune disease or arthritide , in a patient. The methods can include, for example, obtaining a patient sample containing mRNA; analyzing gene expression using the mRNA that results in a gene expression signature of that mRNA, wherein the gene expression signature includes the identification and quantitation of gene expression from genes that have been identified as being differentially expressed in RA; and using that gene expression signature to diagnose or analyze the autoimmune disease or arthritide in said patient, wherein said gene expression of at least about 60% of said genes correlates with that of said gene signature.

[0011] The autoimmune disease or arthritides can be, for example, Rheumatoid Arthritis, Lupus, Ankylosing Spondylitis, fibrositis, fibromyalgia, osteoarthritis, Gout, Juvenile Rheumatoid Arthritis, an autoimmune disease caused by an infectious agent, and the like. Preferably, the autoimmune disease or arthritide can be rheumatoid arthritis. The patient can be, for example, a human, a primate, a dog, a cat, a horse, a sheep, and the like.

[0012] The analysis can be, for example, an analysis of severity of the disease, an analysis of pain manifestation, an analysis of deformity, an analysis of treatment methods, an analysis of treatment efficacy, and the like.

[0013] The gene expression analysis can involve at least about 10 genes that are identified as differentially expressed in arthritis, preferably at least about 50 genes that are identified as differentially expressed in arthritis, more preferably at least about 100 genes that are identified as differentially expressed in arthritis, and the like.

[0014] The genes identified can be expressed at least about 1.5 fold higher or lower than normal, at least about 2 fold higher or lower than normal, at least about 3 fold higher or lower than normal, and the like.

[0015] The genes can include, for example, the 385 genes or ESTs in Table 1 (SEQ ID NOS:1-385), homologs, variant thereof, and the like. The genes can include the genes in cluster A, and in embodiments the genes in cluster A can be down-regulated (SEQ ID NOS:1-37) at least about 2 fold, for example. Further, the genes can include the genes in cluster B, and in embodiments the genes in cluster B can be up-regulated (SEQ ID NOS:1-37) at least about 2 fold

only in late or severe disease, for example. The genes can include the genes in cluster C, and in embodiments the genes in cluster C can be up-regulated (SEQ ID NOS:1-37) at least about 2 fold only in early or mild disease, for example. Also, the genes can include the genes in cluster D, and in embodiments the genes in cluster D can be up-regulated (SEQ ID NOS:1-37) at least about 2 fold in early or mild disease and more in late or severe disease, for example. Furthermore, genes can include the genes in cluster E, and in embodiments the genes in cluster E can be up-regulated (SEQ ID NOS:1-37) at least about 2 fold in both early or mile and late or severe disease, for example.

[0016] Also, the differentially expressed genes can include the 385 genes identified as SEQ ID NOS:1-385, for example. If the genes in clusters B or D are upregulated, the disease can be diagnosed as severe. Furthermore, if the genes in cluster A are upregulated, the disease can be diagnosed as moderate to low-grade.

[0017] Further, the gene expression of at least about 70% of the genes correlates with that of the gene signature, preferably, the gene expression of at least about 80% of the genes correlates with that of the gene signature, more preferably, the gene expression of at least about 90% of the genes correlates with that of the gene signature, still more preferably, the gene expression of at least about 95% of the genes correlates with that of the gene signature, and the like.

[0018] Aspects and embodiments of the invention further provide methods for the treatment of RA that include down-regulating at least one of the genes identified in clusters B through D. Such down-regulation can be achieved by adding antisense oligonucleotides specific for the gene that is being down-regulated, or by adding or expressing a repressor of the gene that is being down-regulated.

[0019] In other embodiments, the invention provides methods for the treatment of RA which involve up-regulating at least one of the genes in cluster A, for example, by adding or expressing a transcriptional activator of the gene that is being up-regulated, or by adding a vector that expresses the protein encoded by the gene that is being up-regulated.

[0020] Further aspects and embodimetns of the invention provide methods for the identification of genes for targeting in the treatment of rheumatoid arthritis in a mammal other than a mouse, which methods involve identifying homologs of SEQ ID NOS:1-385.

[0021] Still other aspects and embodimetns of the invention include methods for the diagnosis of rheumatoid arthritis in a mammal, the methods including obtaining a tissue or fluid sample from a diseased patient; isolating mRNA from said sample; using the isolated mRNA to analyze the gene expression of at least about 40 genes, selected from the group consisting of SEQ ID NOS:1-385 or a homolog thereof, obtaining a fingerprint of the patient's gene expression; and identifying whether at least about 60% of said fingerprint is at least about 2 fold differentially expressed from that of a normal patient.

[0022] Other embodiments include an array or a genechip, specific for rheumatoid arthritis, including at least 10 of the genes selected from the group consisting of SEQ ID NOS:1-

385 or homologs thereof. The array or genechip can include at least 40, 50, 75, 100, or more, of the genes selected from the group consisting of SEQ ID NOS:1-385 or homologs thereof. In some embodiments, the array or genechip consists essentially of such genes, including up to all of the genes of SEQ ID NOS:1-385 or homologs thereof. Such genes can allow for the identification of the severity of the disease, the prognosis of the disease, the diagnosis of the disease, the most efficacious treatment of the disease in a specific patient, and the like.

[0023] In other embodiments, the invention provides methods for the diagnosis or analyses of autoimmune disease or rheumatoid arthritis, including: obtaining mRNA from a patient; using the mRNA as a probe for the analysis of the arrays or genechips disclosed herein; and comparing the results obtained with those of a normal patient.

[0024] Additional embodiments and aspects provide methods of screening the efficacy of a candidate drug *in vitro* for the treatment of collagen-induced arthritis including: identifying vascular endothelial cells expressing FARP mRNA and protein; introducing a candidate drug to said endothelial cells; and evaluating whether said candidate drug causes enhanced or normalized apoptosis of vascular endothelial cells.

[0025] Further, the invention in some embodiments provides methods and materials for reducing the symptoms associated with collagen-induced arthritis including: identifying a subject suffering from collagen-induced arthritis; and administering a compound effective to deplete at least one of the group of FARP mRNA, FARP protein, FARP receptor binding, and FARP activity. Such compound can include, for example, an anti-FARP antibody, capable of interfering with binding of FARP to a FARP receptor.

Brief Description of the Drawings

[0026] **Figure 1.** Hierarchical cluster analysis of 385 genes differentially expressed during CIA. The left panel shows the distribution of gene expression across the hierarchical tree structure in which the values for the first normal sample (1) are set to 1. Rows represent individual genes; columns represent individual values of duplicate samples for each experimental time point. Each cell in the matrix represents the expression level of a single transcript with red and green indicating transcript levels above and below the normal values for that gene across all samples, respectively. The color code for the signal strength in the classification scheme is shown in the box at the bottom left of the panel. Color intensity from pale to deep indicates trust values for the expression of each specific transcript. The colored side bar indicates the five basic clusters of gene expression, with letters corresponding to their grouping. The mean values of all the genes within the indicated groups (A-E) are graphed on the right.

[0027] **Figure 2.** Comparison of microarray and RT-PCR analyses of representative genes in CIA. The patterns of IL-2Rγ and follistatin-like gene mRNA levels, determined by DNA microarray analysis from pooled RNA, are compared to patterns determined by real time RT-PCR analysis of two individual RNA samples.

[0028] **Figure 3.** IL2-Rγ is expressed in the synovial tissue during collagen-induced arthritis. Panel A (dark-field illumination) and panel B (bright-field illumination) show a section through the joint from a normal mouse paw. There is no signal in the joint tissue or surrounding periosteal tissue. Panels C and D show a section through a CIA mouse paw 28 days following primary CII immunization. There is positive signal (bright white grains) in the synovial tissue (arrow) indicating the presence of RNA transcripts for IL2-Rγ. Panels E and F represent a section through the paw of a CIA mouse 49 days following primary CII immunization. There is an extensive chronic inflammatory reaction in the tissue (*) surrounding the cortical bone tissue. Despite the chronic inflammatory reaction in the tissue no significant IL2-Rγ is present in the lesion late in disease. (PO periosteal; CB cortical bone; SY synovium; AS articular surface; Mag 100X)

[0029] **Figure 4.** Tissue-specific expression of differentially regulated genes in lymphoid organs and cells. The presence of specific gene sequences in cDNA libraries generated from the indicated tissues was obtained from the NCBI database using the LocusLink and Unigene databases.

[0030] **Figure 5.** Classification of selected annotated genes. Bars indicate the number of the characterized genes that are involved in the specified biological function (A) or pathway (B). The number of genes in each of the five expression patterns is indicated on each bar. Some genes are represented in more than one category.

Brief Description of Tables 1 and 2

[0031] As mentioned above, filed herewith on two compact discs are two copies of **Table 1**, including **Tables 1.1-1.3**, and **Table 2**, including **Tables 2.1-2.3**. The compact discs are labeled as "Copy 1" and "Copy 2." Each disc has identical content. The contents of the discs are hereby incorporated by reference in their entireties.

[0032] **Table 1** Listing of mouse gene accession numbers, mouse gene name, human mRNA homolog, human protein homologs, and Genbank source of human homolog information. These genes are divided into clusters A through E by expression characteristics as explained herein. Human homologs were identified using unigene and homologene functions at the NCBI database. Further information on the homologous human mRNA sequences can be found in **Table 1.1** under the accession number of interest. Similarly, further information on the homologous human protein sequences can be found in **Table 1.2**, and further information on the "Genbank source" can be found in **Table 1.3**.

[0033] **Table 2** Listing of relevant ESTs. The ESTs are grouped into clusters A through E, as explained herein. Listed are the name of the gene (if known), the accession number of the corresponding homologous human mRNA (if known), the Genbank source number of the human mRNA information, the Genbank accession number for the mouse gene, and a description of similar genes, if known. Further information on the homologous human mRNA sequences

corresponding to the ESTs can be found in **Table 2.1**, under the accession number of interest. Similarly, further information relating to the Genbank source number (human) can be found in **Table 2.2**, and information corresponding to the Genbank accession numbers (mouse) can be found in **Table 2.3**.

Detailed Description of the Preferred Embodiment

[0034] Using microarray analysis, the expression of 8734 cDNAs was analyzed during various stages of mouse CIA, an animal model of RA. From the results, a method for the diagnosis and treatment of RA was developed. Of the 8,734 genes analyzed, 330 were induced and 55 were down-regulated greater than two-fold in early or late diseased paws, as compared to normal paws. Hierarchical clustering resulted in five distinct expression patterns that correlated with histopathologic changes in the paw. Of the 385 genes, the identities of 240 are known. These genes are biologically classifiable into 19 functional categories, the largest being immunity and defense, and into 20 pathway categories, including membrane, secreted and extracellular. Of the known genes, the majority have not been described as playing a role in arthritis. Many of these genes are involved in cell proliferation, differentiation, tumorigenesis, apoptosis, and inflammation. Thus, these global gene expression patterns in diseased paws reveal a large number of genes novel to arthritis, and distinct gene expression profiles distinguishing early and late CIA whose further characterization will advance the understanding of the basic mechanisms responsible for arthritis.

[0035] The results of the analysis of the mouse model of RA include a set of differentially expressed genes that can be used for a variety of purposes. The set of differentially expressed genes can be thought of as a "signature" or a "fingerprint" of RA. Thus, some embodiments of the present invention include DNA arrays or genechips that include one or more of the differentially expressed mouse or human genes identified herein. Further embodiments can include a specific subset of the differentially expressed genes that can represent, for example, genes that are only up-regulated in late disease or genes that are only up-regulated in early disease. A "human Rheumatoid Arthritis genechip" can be used to further study the gene expression of RA as well as other auto-immune diseases, in animal models or in human patients.

[0036] The results of the analysis of the mouse model of RA are also useful in identifying and developing various embodiments of a "human Rheumatoid Arthritis genechip" which includes human homologs of the mouse genes identified herein as well as independently identified genes. The chip and the information obtained can be used to develop methods for diagnosis, prognosis, and analysis of the efficacy of treatments.

[0037] The analysis of mouse genes herein is believed to have covered approximately one third of the genes typically expressed in the mouse genome (a comparable number to that expressed in the human genome). Thus, one embodiment is a method for the identification of other mouse genes involved in RA. In order to thoroughly identify the genes that are differentially expressed in the mouse, arrays or genechips that include a thorough representation of mouse

mRNAs are analyzed using the same method of analysis that identified the RA-specific genes identified herein. However, using the genes identified in the initial analysis of 8734 genes, human or other mammalian homologs can be identified and the differential expression confirmed. The method is also useful for further identifying genes that are up- and down-regulated in human or other mammalian RA and related conditions. Numerous human homologs of the mouse genes are also differentially regulated in human RA comparably to the differential regulation in mouse CIA.

[0038] Thus a method is described herein that identifies the pattern of specific differentially expressed genes, also referred to as the "signature" or "fingerprint" for a particular disease state or a particular patient. The signature is used to diagnose RA in a patient and to analyze the severity of the disease. The pattern of specifically up and down-regulated genes is compared to a "normal" patient, a patient who does not have RA.

[0039] Briefly, genes that are differentially regulated from the normal in patients with RA are identified by any method known to one of skill in the art. With identification of genes involved in the disease and progression of RA, the genetic data are useful in developing a number of methods for use on a patient who has or may have RA or other arthritides.

[0040] Preferred methods involve the identification of the signature of differential expression of one or more of the identified genes for a specific patient. In some embodiments, the method includes isolation of mRNA from a diseased tissue, blood sample, or synovial fluid sample from a patient. The expression of the genes that are specifically identified as differentially regulated is analyzed. The "signature" is produced as the pattern of up and down-regulated genes within that patient's sample. The signature can be used for diagnostic methods, for prognostic methods, for analysis of the most efficacious treatment for the patient, and for analysis of the efficacy of the treatment or the progression of the disease.

Identifying human genes that are differentially regulated in RA

[0041] In some embodiments, the genes that are differentially regulated in human RA are identified by a) using mouse genes associated with CIA to identify human and/or other mammalian homologs thereof using database comparisons, b) using mouse genes associated with CIA to isolate homologs from gene libraries of an animal of interest and/or c) using genes that are known to be involved in mammalian RA and mammalian homologs of those genes.

[0042] In a further embodiment, the genes that are differentially regulated in mammalian RA are identified by microarray analysis using mRNAs from a mammal with RA, using a method comparable to that used herein for identification of the mouse genes. Preferably, the methods identify a thorough representation of the genes involved in RA by one method or another.

[0043] In some embodiments, the mRNAs from the mammal with RA are obtained from a tissue, biological fluid or mixture thereof that contains mRNA. In further embodiments, the mRNAs are isolated from diseased synovial tissue or synovial fluid. In still further embodiments,

the mRNAs are isolated from a blood sample, a saliva sample, or a urine sample. In preferred embodiments, a patient sample is used for which the expression of genes is altered due to the disease.

[0044] Homologs can be genes or DNAs that are 40% similar or more to the mouse genes identified, alternatively, the homologs are at least 50% similar, including 55% similar, 60% similar, 65% similar, 70% similar, 75% similar, 80% similar, 85% similar, 90% similar, 95% similar, and 99% similar. Homologs that are more similar are generally most closely related to the mouse sequence, and thus are in many cases most likely to exhibit similar differential expression in RA. However, the amount of similarity can vary depending on the importance of the region of the gene identified. For example, if the mouse gene is a kinase, the kinase regions are likely to be more homologous or similar then the other regions. The homologs can be DNAs that hybridize under stringent conditions to the mouse genes identified. The stringent conditions under which a homologous gene or DNA will hybridize with the mouse gene can be defined as follows: 0.1X SSPE, 0.1% SDS wash solution at 65°C with 2 washes. (1X SSPE is 180 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA (pH 7.4)). The identification of mammalian homologs can be accomplished using any method known to one of skill in the art. Any genes that have been identified or will be identified as being involved in the disease can be included. Certain genes having a more central or "important" role in different aspects of the disease are thus identifiable. Thus, the subset of genes that are analyzed or contained in a microarray or genechip can be chosen based on the direct or indirect role the gene is found to play in the disease. Alternatively, subsets can be chosen based on what aspect of the disease is being tested. Thus, in some embodiments, those genes that are identified as being involved in "activating" the disease will be included particularly when diagnosis is the desired result. In a further embodiment, those genes that are identified as involved in "progression" of the disease will be included, particularly when treatment, prognosis, or staging of disease is being analyzed. In a further embodiment, those genes involved in remission, regression, or healing of the disease are included, particularly when prognosis, efficacy of treatment, and/or staging of the disease are being analyzed.

[0045] The above method can be altered and applied to all mammals. Thus, in some embodiments, the patient is a mammal. In a further embodiment, the mammal is a human, primate, dog, cat, or horse. Because the incidence of RA in humans is particularly significant, some embodiments include methods for the diagnosis, prognosis and analysis of human RA. Human homologs are identified by methods known to those of skill in the art. In one embodiment, human homologs are identified using computer programs that search for "closest homologs" by inputting the mouse genes and ESTs identified herein. In a further embodiment, the computer analysis can use "active" portions of the sequences or those parts of the gene sequences that are known to be more highly conserved between mammals. The portions that are more highly conserved can be involved in the activity of the protein expressed therefrom. A variety of computer programs can be

used to identify the closest mammalian homologs. In many cases, there can be more than one human homolog that corresponds to the mouse gene.

[0046] In a further embodiment, human homologs are identified by performing the microarray analysis that was used to identify the mouse genes herein. In preferred embodiments, a thorough representation of the human genes that are expressed is analyzed. For example, it is believed that approximately 100,000 genes are actively expressed or included in the human genome. Thus, in order to thoroughly identify those that are involved in the disease RA, a complete representation of the approximately 100,000 genes are analyzed. For example, one or more arrays that contain a thorough representation of the human genome are used to analyze gene expression. In one embodiment, the arrays are from one or more tissues or fluids. In a further embodiment, the arrays are analyzed in duplicate, in triplicate, or in multiple copies. In one embodiment, differential expression can be identified as at least about a 1.4 to 2 fold difference in expression from normal. In a further embodiment, the differential expression is identified as about a 1.6 to 2 fold difference in expression. In a further embodiment, the genes are identified as differentially expressed in RA when there is at least about a 2 fold difference in expression from normal. In a further embodiment, the genes are identified as differentially expressed in RA when there is at least about a 2.3 fold difference in expression from normal. In a further embodiment, the genes are identified as differentially expressed in RA when there is at least about a 2.5 fold difference in expression from normal, including at least about 2.6 fold, 2.7 fold, 2.8 fold, 2.9 fold, 3 fold, 3.5 fold, 4 fold, and 5 fold. However, some genes can show a higher difference in expression than others. These genes can be more involved or alternatively, equally involved in the manifestation of disease as a gene that is less differentially expressed.

[0047] From the above analysis, a "signature" or "fingerprint" can be produced that includes the genes that are differentially expressed in the disease and the range of expression that can be seen among different patients. In one embodiment, the differential expression can be due to different aspects and manifestations of the disease. For example, the fingerprint can be a fingerprint of early RA, late RA, mild RA, extreme RA, RA in remission, a manifestation of RA with little pain, but considerable deformity, a manifestation of RA with considerable pain, but little deformity, etc.

[0048] The expression of many of the genes identified is confirmed using alternative methods known to one of skill in the art, including Northern blotting, quantitative PCR techniques such as real-time PCR, or other methods of expression analysis. Alternatively, the translation products and expression can be analyzed by methods known to one of skill in the art, such as Western blotting, activity assays, etc.

[0049] In a further embodiment, the genes identified as part of the "signature" or "fingerprint" are further analyzed as to their involvement in the disease. In one embodiment, a gene is further analyzed by any method known to one of skill in the art and can identify the involvement

in activation, progression, pain manifestation, deformation, and treatment of the disease. Patients that express certain genes or subsets identified above will often show a greater response to certain types of treatments then others. For example, if one patient expresses high amounts of IL-2, that patient would respond better to treatments that target IL-2 activity, expression, or the downstream effects of IL-2.

[0050] One embodiment of this "signature" or "fingerprint" is an array or a genechip that includes the genes that are identified as differentially expressed in one or all manifestations of RA, which can be referred to as a "human Rheumatoid Arthritis genechip." A variety of genechips can be produced that are specific to different aspects of the disease. In one embodiment, a genechip can be produced with only those genes that are identified as possessing key roles in each aspect of the disease. In a further embodiment, a genechip can be produced that includes only those genes that are expressed late in disease or in severe disease.

Method of diagnosis, prognosis, and treatment analysis of a patient with rheumatoid arthritis

[0051] The genes that are identified above as being involved in RA can be analyzed as to differential expression in a specific patient by any means known to one of skill in the art. Some embodiments involve isolation of the mRNA from a patient sample.

[0052] Briefly, mRNA is isolated from at least one tissue or sample from the patient. In one embodiment, the sample is a diseased tissue sample, including but not limited to synovial tissue. In a further embodiment, the sample is a fluid containing disease cells or mRNA, including, but not limited to, synovial fluid, and blood.

[0053] The mRNA can then be used to analyze gene expression by any method known to one of skill in the art. In one embodiment, the mRNA is used to analyze a "human Rheumatoid Arthritis genechip" or array. From this analysis, a specific patient "signature" of the genes and amount of differential expression is produced. The amount of differential expression is compared to a normal patient. In one embodiment, the ranges and values of expression for a normal patient are derived using at least 2 normal patients, including at least 3, at least 4, at least 5, at least 10, at least 20, and at least 50. In a further embodiment, the ranges and values of expression for a normal patient are derived using a statistical sampling of the population, or a statistical sampling of the area, ethnic group, age group, social group, or sex. In a further embodiment, the range and values of gene expression for a normal patient are derived from the patient before disease or during remission.

[0054] The results of the signature can be used in any one or more of the methods disclosed herein. Alternatively, one or more of the analyses can be included in one chip or array. The specific signature can include the results of the expression levels of one or more genes in that specific patient. In one embodiment, the signature is the results of the expression levels of at least 10 genes, preferably 40 genes, however, the signature can include the results of 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, 750, 1000, 2000, 5000, and 10, 000 genes which have been identified as

being differentially expressed in RA. Some genes are more important or more involved in the manifestation or activation of the disease. Thus, the signature can require fewer genes when those that are more important have been identified and included.

[0055] In one embodiment, the results of the signature are used in a method of diagnosis. The method of diagnosis can include, a method of diagnosis of rheumatoid arthritis, a method of diagnosis of severity of the disease, a method of diagnosis of a manifestation of the disease and can include any or all of the above. Many of the same genes that are differentially expressed or involved in the manifestation of RA can also be involved in a different autoimmune disease. Alternatively, many of the same genes that are differentially expressed or involved in the manifestation of RA can also be involved in a different arthritide. Thus, the method of diagnosis can diagnose an arthritic or autoimmune disease, including, but not limited to, Lupus, Juvenile RA, Ankylosing Spondylitis, gout, osteoarthritis, fibrositis and fibromyalgia, Scleroderma, and even the autoimmune manifestations of Lyme disease and *Streptococcus* infection.

[0056] In a further embodiment, the results of the signature can be used in a method for prognosis of disease. The prognosis in various patients can vary tremendously. Some patients may progress very rapidly and may need a very aggressive treatment plan. Other patients may have a very mild version and may progress very slowly, requiring a more subtle treatment plan. This can be important when considering side effects, quality of life, and patient needs.

[0057] In a further embodiment, the results of the signature are used in a method of identification of the most efficacious treatment for that specific disease and for that specific patient. The treatment and the response to a drug can depend on which genes are being expressed. For example, in its most simple form, a patient with little IL-2 expression would not be best treated using a treatment that targets IL-2. However, the choice of a treatment method can involve a number of factors besides the gene expression of specific genes, including, the form of the disease, the severity of the disease, the manifestation of the disease, and the needs and wants of the patient. Many of these factors can be identified using one of the methods included herein.

[0058] In a further embodiment, the results are used to identify single nucleotide polymorphisms (SNPs), mutations, or Restriction Fragment Length Polymorphisms (RFLPs) associated with RA or other autoimmune diseases or other arthritides. The genes that are identified can be included in one or all of the genechips, arrays or analyses herein. In an alternative embodiment, a genechip that includes single nucleotide polymorphisms (SNPs), mutations, or Restriction Fragment Length Polymorphisms (RFLPs) is produced and used for diagnosis, prognosis, and/or identification of the best treatment or drug for use in treating RA.

Method of Identifying Targets for Drugs

[0059] In a further embodiment, the results of the signature are used to identify drug targets. Any or all of the genes identified herein and included in the signature or on a rheumatoid

arthritis array can be used to further identify drugs or treatments that would target that gene or gene product.

[0060] Methods of identifying targets can include any method known to one of skill in the art, including, but not limited to: producing and testing small molecules, oligonucleotides (including antisense, RNAi and triplex formers), antibodies, and drugs that target any of the genes or gene products identified herein. Alternatively, gene therapy can be used to down-regulate, up-regulate, or express proteins or gene products identified herein.

[0061] The present methods will be further described by use of the following examples.

EXAMPLES

[0062] In some of the following examples, the paws of mice with collagen-induced arthritis were analyzed in early disease and late disease by isolation of the RNA and microarray analysis. The results were confirmed using RT-PCR and *in situ* hybridization. Down- and up-regulation of genes was identified and the genes were clustered into groups. Human homologs are identified and the expression patterns are used to diagnose RA, to analyze the severity of disease in a patient, and to identify new treatments for arthritis. A number of genes were identified that previously had not been identified as being involved in arthritis; the genes thus identified can represent gene targets for drug therapy.

[0063] In the Examples relating to mouse experiments, DBA/1 mice were immunized with type II bovine collagen to induce arthritis, and mRNA was isolated from paws of non-immunized mice and from severely affected paws of mice at 28 days (acute disease model) and 49 days (chronic disease model) following the primary collagen injection. A single common reference control was used for all microarrays consisting of mRNA derived from the whole of a postnatal day 1 mouse, and all mRNAs were hybridized to duplicate microarrays (Incyte Pharmaceuticals, Inc., Palo Alto, CA). Among the 385 disease-specific genes differentially regulated in CIA are 102 expressed sequence tags (ESTs). Microarray analyses will help in further mapping out differences in gene expression between normal synovium and the synovium of acute and chronic CIA, including the identification of novel genes involved in arthritis.

Example 1

Production of mice with collagen-induced arthritis (CIA)

[0064] Mice with collagen-induced arthritis were used as a model for RA. Male DBA/IJ mice, 6 to 8 weeks of age, were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were housed in the animal care facility at The Children's Hospital Research Foundation (Cincinnati, OH) under Institutional Animal Care and Use Committee approved conditions. Arthritis was induced with bovine type II collagen (CII, Elastin Products Co., Owensville, MO), as

previously described (Thornton, *et al. J. Immunol* (2000) 165:1557-1563). Briefly, mice were injected intradermally with 100 μg of CII in complete Freund's adjuvant (CFA) at the base of the tail on day 0, and a similar booster was administered on day 21. Mice were evaluated for arthritis using an established macroscopic scoring system ranging from 0 to 4 (0 = no detectable arthritis, 1 = swelling and/or redness of paw or one digit, 2 = two joints involved, 3 = three or four joints involved and 4 = severe arthritis of the entire paw and digits). At day 28 (early disease) and day 49 (late disease) following primary immunization, mice were sacrificed. Hind paws with an arthritic score of four were removed for mRNA analysis and *in situ* hybridizations (ISH). Paws from mice of the same age not treated with CII were used as normal controls.

Example 2

mRNA expression profiling of early and late CIA

[0065] Differential gene expression in paws of mice with CIA was analyzed in early (day 28) and late (day 49) arthritis and compared to that of paws from normal mice. These time points were chosen based on earlier studies that demonstrated their correlation with distinct histologic appearance and mRNA expression patterns by RPA.

[0066] RNA was isolated from paws that were quick frozen in liquid nitrogen and stored at -80°C. Frozen paws were minced with a scalpel and homogenized with a Polytron Tissue Tearor (Biospec Products, Bartlesville, OK) in appropriate volumes of RNA Stat-60 (Tel-Test, Friendswood, TX). Total RNA was extracted from the tissue homogenates according to the manufacturer's instructions. Pooled total RNA from normal (4 paws), early arthritic (3 paws) and late arthritic (4 paws) paws was used to isolate polyA+ RNA by the Oligotex mRNA isolation kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. RNA concentrations were measured by fluorometry using the Ribogreen RNA Quantification Kit (Molecular Probes, Inc., Eugene, OR).

[0067] DNA microarray analysis was performed as follows: mRNA of a whole 1 day old mouse was used for normalization of gene expression levels across all six microarray chips. Competitive hybridizations with Cy3 labeled whole 1 day old mouse mRNA versus Cy5 labeled normal paw mRNA, Cy5 labeled early paw mRNA or Cy5 labeled late paw mRNA were performed. Each sample (normal, early and late) was labeled and hybridized to two microarray chips. Hybridizations were performed on the mouse GEM1 array by Incyte Genomics (Palo Alto, CA).

[0068] Primary data were examined using Incyte Gemtools software and GeneSpring version 4.0.4 software (Silicon Genetics, Redwood City, CA). Defective cDNA spots (irregular geometry, scratched, or <40% area compared to average) or spot fluorescence hybridizations with signal to noise ratios less than 2.5:1 were eliminated from the data set. Data sets were subjected to normalization first within each microarray experiment such that the median of the Cy5 channel was

balanced against the ratio of the Cy3 channel (k*(MedianCy3) = MedianCy5, where k is the ratio of the median intensities in each). Each microarray contained control genes present as non-mammalian single gene "spikes" or "complex targets". The complex targets consisted of probe-sets that contain a pool of cellular genes expressed in most cell types. In addition, each experimental mRNA sample was augmented with incremental amounts of non-mammalian gene RNA (2X, 4X, 16X, etc) to permit assessment of the dynamic range attained within each microarray. Little variation was observed across the microarray series with respect to the 192 control genes (not shown), providing support for inter-array comparisons of temporally regulated genes. Genes were clustered according to their expression pattern by subjecting the log-transformed data ($R= \log_2 Cy5/(kCy3)$, where R is the log of the expression ratio for each gene) to the hierarchical tree clustering algorithm as implemented in the GeneSpring program (Silicon Genetics). The hierarchical tree analysis was performed using a minimum distance value of 0.001, separation ratio of 0.5 and the standard correlation distance definition.

[0069] Mouse sense and antisense RNA probes were synthesized using the Stratagene RNA Transcription Kit (Stratagene, La Jolla, CA). T3 or T7 RNA polymerase produced ^{35}S-radiolabeled antisense or sense single-stranded RNA probes, respectively. A sense probe generated from an unrelated mouse gene was used as a negative control for *in situ* hybridization.

[0070] For early and late disease, mRNA from paws with severe arthritis (score of 4) were used to generate probes that were hybridized to Incyte Mouse GEM1 chips, as was mRNA from normal mouse paws. Hybridizations were conducted on duplicate chips, allowing for the elimination of genes whose expression levels differed by greater than 50% between the duplicate samples. 8,734 cDNAs, including known genes and ESTs, were represented on the microarray chip. 385 genes exhibited a greater than two-fold difference in expression between arthritic and normal paws and were selected for further analysis. Expression of 304 of these genes differed only between arthritic and normal paws, and expression of 81 of these genes differed between early and late arthritis. However, some of the genes identified were duplicates. Thus, the genes listed in Table 1 include some duplicates.

[0071] Figure 1 demonstrates the 385 selected genes and their average levels of expression as compared to normal tissue values. The majority of genes were more highly expressed in arthritic paws as compared to normal paws. Genes were clustered according to their expression pattern during disease by hierarchical tree analysis. The resulting hierarchical tree structure revealed five distinct patterns of expression. Approximately half of the genes, represented by clusters D and E in Table 1 (225 genes, 58.4%), were upregulated both in early and late disease. It was possible to separate these genes into those with similar expression levels in early and late disease (cluster E in Table 1) and genes whose expression levels further increased during late disease (cluster D in Table 1). These may represent two distinct patterns or a continuum of coordinately regulated gene groups. Cluster C in Table 1 (105 genes, 27.3%) represents genes

principally upregulated in early disease. Cluster B in Table 1 (18 genes, 4.7%) represents genes predominantly upregulated in late disease. Cluster A in Table 1 (37 genes, 9.6%) represents genes downregulated during both early and late disease, compared to normal paws. The individual genes and the number of ESTs belonging to each cluster are listed in Table 1. Please see Table 2 for the EST accession number and Table 3 for a schematic representation of the characteristics of Clusters A through E.

Table 1 Sequences – Human Homologs and Accession Numbers

Cluster A:

Mouse #	Name (mouse)	Human mRNA #	Human Protein #	Genbank Source
W09829	trefoil factor 2 (spasmolytic protein 1)	NM_005423	NP_005414	AH003622
W36838	uteroglobin	NM_003357	NP_003348	BC004481
AA028678	palate, lung, and nasal epithelium expressed transcript	NM_016583	NP_057667	BC012549
AA047966	four and a half LIM domains 1	NM_001449	NP_001440	BC010998
AA108401	solute carrier family 27 (fatty acid transporter)	NM_003645	NP_003636	D88308
AA145089	potassium voltage-gated channel, subfamily H, member 2	NM_000238	NP_000229	U04270
AA241859	betaine-homocysteine methyltransferase	NM_001713	NP_001704	U50929
AA271284	myoglobin	NM_005368	NP_005359	X00371,X00372 X00373
AA261313	nuclear receptor subfamily 1, group H, member 4	NM_005123	NP_005114	U68233
AA275042	amine N-sulfotransferase	NM_001054.1	NP_001045	59% homologous
AA268120	cytochrome P450, steroid inducible 3a11	NM_007818	NP_001045	X 60452
AA501052	cardiac morphogenesis			62% homologous AW755250

Cluster B:

Mouse #	Name (mouse)	Human mRNA #	Human Protein #	Genbank Source
W11965	*enolase 3, β muscle*	NM_001976	NP_001967	X51957,X56832
W64550	*tumor-associated calcium signal transducer 2*	NM_002353	NP_002344	X77753
AA388939	IG α chain C region	NM_001810	NP_001801	AL109804
W34420	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	NM_004320	NP_004311	AH005190
AA015155	S100 calcium binding protein A3	NM_002960	NP_002951	Z18948
AA204246	Mus musculus dystonin (Bpag1-n)	NM_001723	NP_001714	L11690,M69225
W62819	neuronal protein 3.1	NM_004772	NP_004763	U30521
W64937	*angiopoietin related protein 2*			AF007150, AI467954, AI 081

Cluster C:

Mouse #	Name (mouse)	Human mRNA #	Human Protein #	Genbank Source
W82159	Fc receptor, IgG, low affinity III	NM_000570	NP_000561	X16863
AI894016	complement component 1, q subcomponent, c	XM_031238	XP_031238	AK057792, BC009016
AI324436	Phospholipase A2 group VII	NM_005084	NP_005075	U20157
AA116505	CD53 antigen	NM_000560	NP_000551	AH011005,M37033
AA177717	Interleukin 1 receptor, type I	NM_000877	NP_000868	M27492
AI322933	Interleukin 4 receptor, α	NM_000418	NP_000409	X52425
AA220007	CD68 antigen	NM_001251	NP_001242	S57235
AA289476	Chemokine (C-C) receptor 2	NM_000647	NP_000638	U03882
AA289559	Ecotropic viral integration site 2	NM_014210	NP_055025	AH002689
AI508758	CD14 antigen	NM_000591	NP_000582	X13334
AA286506	Interleukin 4 receptor, α	NM_000418	NP_000409	X52425
AA467489	Integrin β 2 (Cd18)	NM_000211	NP_000202	M15395
AA423373	Glycoprotein 49 A	NM_024318	NP_077294	AF041262
AA435060	Leucocyte specific transcript 1			LY117
AA475774	Cathepsin C	NM_001814	NP_001805	AU076460,X87212
AA497620	small proline-rich protein 2A	NM_005988	NP_005979	X53064
W11889	Hemochromatosis	NM_000410	NP_000401	U60319
W13905	Fibrinogen/angiopoietin-related protein	NM_016109	NP_057193	AF153606,AF202636
W96914	lysyl oxidase	NM_002317	NP_002308	AF039290,AF039291
AA080231	mannosidase 2, α B1	NM_000528	NP_000519	AH006687
AA152885	small inducible cytokine subfamily B (Cys-X-Cys)	NM_006419	NP_006417	47% homologous AF044197
AA170386	colony stimulating factor 2 receptor, β 2, low-affinity	No human genes		
AA201097	protein tyrosine phosphatase, receptor type, C	NM_002838	NP_002829	Y00062,Y00638
AA197349	baculoviral IAP repeat-containing 2	NM_001166	NP_001157	L49431,U37547
AA239171	elastin	NM_000501	NP_000492	AH007100
AA268708	Mus musculus hypoxia induced gene 2 (Hig2)	No human genes		
AA259959	CD37 antigen	NM_001774	NP_001765	X14046
AA260521	uncoupling protein 2, mitochondrial	NM_003355	NP_003346	AF096289
AA274104	interleukin 2 receptor, γ chain	NM_000206	NP_000197	D11086
AA387058	apoptotic protease activating factor 1	NM 013229	NP 037361	
AA547555	CDC28 protein kinase 1	NM_001826	NP_001817	X54941
AA140523	Rac GTPase-activating protein 1	NM_013277	NP_037409	AL136794
AA521764	receptor (calcitonin) activity modifying protein 2	NM_005854	NP_005845	AJ001015
AA051654	metallothionein 1	M 10942	AAA5999587	85% homologous
AA265259	oncostatin receptor	NM_003999	NP_003990	U60805
AA178121	cathepsin S	NM_004079	NP_004070	AL356292,BC002642, BQ006623,M90696
AA210306	a disintegrin and metalloproteinase domain 9	NM_003816	NP_003807	U41766
AA230451	S100 calcium binding protein A8 (calgranulin A)	NM_002964	NP_002955	A12027,Y00278
AA268219	macrophage expressed gene 1	No human genes		
W41459	Eukaryotic translation initiation factor 1A	NM_001412	NP_001403	L18960
AA003549	Homolog of human ftp-3	NM_003011	NP_003002	M93651

Mouse #	Name (mouse)	Human mRNA #	Human Protein #	Genbank Source
AA063753	ATP-binding cassette, sub-family A (ABC1), member 1	NM_005502	NP_005493	AF165281,AF275948
AI594919	Intersectin (SH3 domain protein 1A)	NM_003024	NP_003015	AF064244
AI385509	Nuclear factor of κ light polypeptide enhancer p49/p100	NM_002502	NP_002493	X61498
AA087193	Lipocalin 2	NM_005564	NP_005555	X99133
AA175094	Myristoylated alanine rich protein kinase C substrate	NM_002356	NP_002347	D10522
AI451276	SH3 domain protein 3	NM_012383	NP_036515	BC007459
AA209640	Histocompatibility 2, complement component factor B	NM_001710	NP_001701	BC004143,L15702
AA276440	Selenoprotein P, plasma, 1	NM_005410	NP_005401	Z11793
AA432934	Neuropilin	NM_003873	NP_003864	AF018956
AA499926	Peptidylprolyl isomerase A	NM_021130	NP_066953	X52851
AA538499	Phosphatidylinositol-4-phosphate 5-kinase, type II, α	NM_005028	NP_005019	BC018034
AA414612	Capping protein α1	NM_006135	NP_006126	U56637
W42321	Pentaxin related gene	NM_002852	NP_002843	X63613
AA162537	Type II transmembrane protein MDL-1	NM_013252	NP_037384	AJ271684
AI646186	Schlafen 4	NM_018042	NP_060512	41% homologous
AA462202	BP-3 alloantigen	NM_004334	NP_004325	D21878
AI322278	Pyruvate dehydrogenase kinase 4	NM_002612	NP_002603	U54617
W98241	proprotein convertase subtilisin/kexin type 5	NM_006200	NP_006191	BC012064
AA172456	small inducible cytokine A12	NM_006273	NP_006264	X71087
AA178155	small inducible cytokine A4	NM_002984	NP_002975	J04130
AA267811	lymphocyte cytosolic protein 2	NM_005565	NP_005556	U20158
AA144482	chemokine (C-C) receptor 5	NM_000579	NP_000570	AH005786
AA266002	B-cell leukemia/lymphoma 3	NM_005178	NP_005169	M31732

Cluster D:

Mouse #	Name (mouse)	Human mRNA #	Human Protein #	Genbank Source
AA064293	cartilage oligomeric matrix protein	NM_000095	NP_000086	L32137
AI552105	alkaline phosphatase 2, liver	NM_000478	NP_000469	AB011406,AH005272
AA145458	fibronectin 1	NM_002026	NP_002017	M15801,X02761
AA177218	IG α chain C region	NM_001810	NP_001801	AL109804
W63981	fibromodulin	NM_002023	NP_002014	U05291,X75546
AA030995	peptidylprolyl isomerase B	NM_012117	NP_036249	S62077
AA241281	aquaporin 1	NM_000385	NP_000376	M77829,U41517
AA260949	growth arrest and DNA-damage-inducible, γ	NM_006705	NP_006696	AF079806,AF265659
AA518165	tissue inhibitor of metalloproteinase 2		A37128	100% homologous
W33786	procollagen, type VI, α 1	NM_001848	NP_001839	M20776,X15879,
AA000107	procollagen, type XI, α 2	NM_080679	NP_542410	AH006115,U32169
AI323131	thrombospondin 3	NM_007112	NP_009043	L38969
AA073904	dickkopf homolog 3 (Xenopus laevis)	NM_013253	NP_037385	AF177396
AA109900	*hemoglobin α, adult chain 1*		P01922	85% homologous
AA537116	immunoglobulin superfamily with leucine-rich repeats	NM_005545	NP_005536	AB003184
AI327504	eukaryotic translation elongation factor 1 α 2	NM_001958	NP_001949	X70940

Cluster E:

Mouse #	Name (mouse)	Human mRNA #	Human Protein #	Genbank Source
W13151	thymus cell antigen 1, τ	NM_006288	NP_006279	AL161958
W54287	Biglycan	NM_001711	NP_001702	AH002674,BC002416
W89883	procollagen, type III, α 1	NM_000090	NP_000081	AI755052,M26939,X14420
AA175226	complement component 1, r subcomponent	NM_001733	NP_001724	X04701
AA242149	FK506 binding protein 7 (23 kDa)	NM_017946	NP_060416	
AA209006	complement component 1, s subcomponent		P09871	75% homologous
AA270625	tenascin C	NM_015904	NP_056988	AF078035,AJ006776
AA538511	histocompatibility 2, L region		S48134	69% homologous
W10072	insulin-like growth factor 1	NM_000618	NP_000609	X57025
W14393	Sid394p	NM_006815	NP_006806	BC 025957
W11571	hexokinase 1	NM_022361	NP_071756	BC022323
W14289	cathepsin Z	NM_001336	NP_001327	AF136273
W16254	tubulin, β 5	NM_001069	NP_001060	X79535
W17813	Talin	NM 006289	NP 006280	
W82677	bone morphogenetic protein 1	NM_001199	NP_001190	M22488
W83904	peptidylprolyl isomerase C	NM_000943	NP_000934	BC002678
W89354	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	NM_001084	NP_001075	BC011674
W99856	procollagen, type V, α 1	NM_000093	NP_000084	D90279,L38808,M76729
AA002439	annexin A5	NM_001154	NP_001145	AH004914,J03745
AA030294	Mus musculus frizzled-1	NM_003505	NP_003496	AB017363
AA030780	peroxisomal δ3, δ-2-enoyl-Coenzyme A isomerase	NM_006117	NP_006108	AF153612
AA038395	Ras suppressor protein 1	NM_012425	NP_036557	L12535
AA060268	phospholipase D3	NM_012268	NP_036400	U60644
AA067258	Calumenin	NM_001219	NP_001210	AF013759,U67280
AA110872	amyloid β (A4) precursor protein	NM_000484	NP_000475	AH005295
AA118715	CD97 antigen	NM_001784	NP_001775	X84700
AA122791	histocompatibility 2, Q region locus 7		I37519	68% homologous
AA222201	butyrate response factor 1	NM_005141	NP_005132	J00129,M64983
AA242611	follistatin-like	NM_007085	NP_009016	BC000055
AA397114	annexin A4	NM_001153	NP_001144	D78152,M82809
AA259366	Trp4-associated protein TAP1	NM_015638	NP_056453	BC013144
AA259551	eukaryotic translation elongation factor 1 α 1	NM_006452	NP_006443	BC010273
AA271275	metallocarboxypeptidase CPX-1	NM_019609	NP_062555	
AA260248	growth factor receptor bound protein 10	NM_005311	NP_005302	D86962
AA437882	ribosomal protein L9	NM_000661	NP_000652	BG829769,U09953
AA396298	RNAse 4	NM_002937	NP_002928	BC015520
AA474964	Lactotransferrin	NM_002343	NP_002334	X53961
AA499296	annexin A6	NM_001155	NP_001146	J03578,X77673
AA547428	protein kinase, cAMP dependent, catalytic, β	NM_002731	NP_002722	M34181
W18828	dihydropyrimidinase-like 3	NM_001387	NP_001378	D78014
AA048915	guanine nucleotide binding protein, β-2, related sequence 1	NM_003922	NP_003913	U50078
W14837	protease, cysteine, 1	NM_005606	NP_005597	BC003061
W18376	golgi vesicular membrane trafficking protein p18	NM_005868	NP_005859	AF007551
W80177	matrix metalloproteinase 2	NM_004530	NP_004521	AH002654
AA003452	thrombospondin 4	NM_003248	NP_003239	Z19585

AA073604	procollagen, type I, α 1	NM_000088	NP_000079	Z74615
AA124340	transforming growth factor, β receptor III	No human genes		
AA241784	insulin-like growth factor binding protein 5	NM_000599	NP_000590	
AA268082	Lumican	NM_002345	NP_002336	BC007038
AA260280	procollagen, type III, α 1	NM_000090	NP_000081	AI755052,M26939,X14420
W13698	FK506 binding protein 9	NM_007270	NP_009201	BC011872
W14113	twist gene homolog, (Drosophila)	NM_000474	NP_000465	U80998,X99268
AA052081	Atpase, class I, type 8B, member 2	No human genes		
W89518	annexin A2	NM_004039	NP_004030	D00017
AI894006	procollagen, type XI, α 1	NM_001854	NP_001845	AU118365,J04177,U12139
AA002481	integrin β 5	NM_002213	NP_002204	BC006541
AA023549	procollagen, type V, α 2	NM_000393	NP_000384	BC015705,M58529,Y14690
AA033050	serine protease inhibitor 4	NM_006216	NP_006207	BC015663
AA037995	microfibrillar associated protein 5	NM_003480	NP_003471	AH007047
AA059524	procollagen, type VI, α 3	NM_004369	NP_004360	X52022
AA066921	integral membrane protein 2	NM_004867	NP_004858	AF038953
AA108363	ribosomal protein L3	NM_000967	NP_000958	BC008492,BC012146
AA108928	secreted phosphoprotein 1	NM_000582	NP_000573	AF052124
AA220699	transcobalamin 2	NM_000355	NP_000346	AF047576,M60396
AA272097	fibroblast growth factor receptor 1	NM_000604	NP 000595	M34641,X66945
AA451495	protocadherin 13	NM_003735	NP_003726	
AA509765	Endomucin	NM_016241	NP_057325	
AA542013	fibroblast growth factor receptor 1	NM_000604	NP_000595	M34641,X66945
AA047991	keratin complex 2, basic, gene 1	NM_001004	NP_000995	BC005354,BC005920,BC007573
W17771	cathelin-like protein	NM_004345	NP_004336	Z38026
AA221044	histocompatibility 2, L region		S48134	69% homologous
AI322868	myristoylated alanine rich protein kinase C substrate	NM_002356	NP_002347	D10522
AA024088	SH3 domain protein 3	NM_012383	NP_036515	BC007459
W18121	histocompatibility 2, complement component factor B	NM_001710	NP_001701	BC004143,L15702
AA266975	cell division cycle 42 homolog (S. cerevisiae)	NM_001791	NP_001782	AL121735,BC003682,M57298
AA172527	ATP-binding cassette, sub-family G, member 1	NM_004915	NP_004906	X91249
AA175651	caspase 11	NM_004347	NP_004338	U28015
AA260476	calpain 6	NM_014289	NP_055104	AL031117
W98807	FXYD domain-containing ion transport regulator 5	NM_014164	NP_054883	AA044211,AA296696,AF161462,BG025158
AA068750	stromal cell derived factor 1	NM_000609	NP_000600	U16752
AA109951	β-2 microglobulin	NM_004048	NP_004039	AB021288
AA200339	secretory leukocyte protease inhibitor	NM_003064	NP_003055	M74444,X04470
AA245698	regulator of G-protein signaling 5	NM_003617	NP_003608	AB008109
AA268592	transforming growth factor, β induced, 68 kDa	NM_000358	NP_000349	M77349
AA272807	histocompatibility 2, class II antigen A, α	NM_002122	NP_002113	L34083,L46875,M20431
W10023	catenin β	NM_001904	NP_001895	X87838
W12260	surfeit gene 4	NM_017503	NP_059973	BC014411,BM789997

W14138	kallikrein 3, plasma	No human genes		
W14540	histocompatibility 2, K region		P18462	68% homologous
W34612	transglutaminase 2, C polypeptide	NM_004613	NP_004604	M55153
W64075	proline rich protein expressed in brain	NM_014764	NP_055579	D31767
W81878	osteoblast specific factor 2	NM_006475	NP_006466	D13666
W82141	lysosomal membrane glycoprotein 1	NM_005561	NP_005552	J04182
W82946	benzodiazepine receptor, peripheral	NM_000714	NP_000705	AH000829,M36035,U12421
AA119072	ceroid-lipofuscinosis, neuronal 2	NM_000391	NP_000382	AF039704
AA123008	membrane bound C2 domain containing protein	NM_015292	NP_056107	BC004998
AA137942	immunoglobulin J chain precursor		P01591	77% homologous
AA172867	purine-nucleoside phosphorylase	NM_000270	NP_000261	X00737
AA178779	interferon concensus sequence binding protein	NM_002163	NP_002154	M91196
AA185869	β-1,4 N-actylgalactosaminyltransferase	NM_001478	NP_001469	L76079,M83651
AA209884	guanine nucleotide binding protein (G protein), γ 10	NM_004125	NP_004116	BC015391
AA241132	coatomer protein complex, subunit γ 1	NM_016128	NP_057212	AF100756
AA230649	histocompatibility 2, class II, locus Dma	NM_006120	NP_006111	X62744
AA260654	TG interacting factor	NM_003244	NP_003235	X89750
AA268148	eukaryotic translation elongation factor 1-β homolog	NM_007086	NP_009017	AJ006266
AA396152	CD44 antigen	NM_000610	NP_000601	AJ251595
AA271576	receptor (TNFRSF)-interacting serine-threonine kinase 1	NM_003804	NP_003795	U50062
AA276030	ATPase-like vacuolar proton channel	NM_001694	NP_001685	BC009290,BI548787
AA414089	heterogeneous nuclear ribonucleoprotein D-like	NM_014740	NP_055555	D21853
AA413831	p100 co-activator	NM_014390	NP_055205	U22055
AA060205	butyrate response factor 1	NM_005141	NP_005132	J00129,M64983
AA200393	CTP synthetase homolog	NM_019857	NP_062831	AK024070
AA416325	ATX1 (antioxidant protein 1) homolog 1 (yeast)	NM_004045	NP_004036	U70660
AA198703	apoptotic protease activating factor 1	NM_001160	NP_001151	AF013263
AA457927	polypeptide N-acetylgalactosaminyltransferase 1	NM_020474	NP_065207	U41514,Y10343

Table 2: ESTs

Cluster A

Mouse gene name	Human mRNA	Genbank Source	Genbank	Description
no known gene	no homologene		AA217294.1	Public domain EST {IMAGE:653016}
no known gene	no homologene		W41083	ESTs, Weakly similar to AF127035_1 calcium-activated chloride channel protein 2 [H.sapiens]
no known gene	no homologene		AA137298	ESTs
no known gene	no homologene		AA268133	ESTs
no known gene	no homologene		AA027728.1	Public domain EST {IMAGE:463464}
no known gene	no homologene		AA209551	ESTs
no known gene	no homologene		AA395994	ESTs
tridadin	NM_006073	U18985	AA466026	ESTs, Moderately similar to triadin [H.sapiens]
no known gene	no homologene		AA080287	ESTs
extracellular link domain containing 1	NM_006691	AF118101	AA269330	ESTs, Moderately similar to AF118108_1 lymphatic endothelium-specific hyaluronan receptor LYVE-1 [H.sapiens]
no known gene	no homologene		AI450674	ESTs, Moderately similar to T20D3.3 [C.elegans]
no known gene	no homologene		AA290313	ESTs
retinoblastoma-associated protein HEC	NM_006101	AF017790	W99015	ESTs, Moderately similar to retinoblastoma-associated protein HEC [H.sapiens]
no known gene	no homologene		AA288562.1	Public domain EST {IMAGE:749337}
no known gene	no homologene		AI595209	ESTs
RAP 1 GTPASE activating protein 1	NM_ 002885	M64788	AI509969	ESTs, Highly similar to RAP1 GTPASE ACTIVATING PROTEIN 1 [Homo sapiens]
syaptotogmin 1	NM_005639	M55047	W15872	ESTs
cystaithionine gamma lyase	NM_001902	S52028	AA245993	ESTs, Highly similar to CYSTATHIONINE GAMMA-LYASE [Homo sapiens]
tocopherol alpha transfer protein	NM_000370	D49488	AA277652	ESTs
no known gene	no homologene		AA061834	ESTs
ectodermal neural cortex	NM_003633	AF059611	AI608121	ESTs, Weakly similar to open reading frame [M.musculus]
no known gene	no homologene		AA145023	ESTs
no known gene	no homologene		AA414733	ESTs
no known gene	no homologene		AA259388	ESTs
no known gene	NM_017779	AK000361	AA254513	ESTs

Cluster B

Mouse gene name	Human mRNA	Genbank Source	Genbank	Description
no known gene	no homologene		W09957	ESTs, Moderately similar to unnamed protein product [H.sapiens]
no known gene	no homologene		W33467	ESTs
myosin binding protein C	NM_004533	X73113	AI385497	ESTs, Moderately similar to C-PROTEIN, SKELETAL MUSCLE FAST-ISOFORM [Gallus gallus]
Latenet transforming growth factor beta binding protein 4	NM_003573	Y13622	AA268327.1	Public domain EST {IMAGE:733726}
aggrecan 1	NM_001135	M55172	AA396306.1	Public domain EST {IMAGE:803275}
no known gene	no homologene		AA066452	ESTs, Weakly similar to A45910 ultra-high-sulfur keratin - mouse [M.musculus]
human retinoic acid receptor responder protein 1	NM_002888	U27185	AI464827	ESTs, Weakly similar to TIG1_HUMAN RETINOIC ACID RECEPTOR RESPONDER PROTEIN 1 [H.sapiens]
no known gene	no homologene		AA038095.1	Public domain EST {IMAGE:472860}
no known gene	no homologene		AA038926	ESTs
creatine kinase	NM_001825	J05401	AI322288	ESTs, Highly similar to CREATINE KINASE, SARCOMERIC MITOCHONDRIAL PRECURSOR [Rattus norvegicus]

Cluster C

Mouse gene name	Human mRNA	Genbank Source	Genbank	Description
no known gene	NO HUMAN CDNA		AA221886	ESTs
TNFa induced adipose-related protein	NO HUMAN CDNA		AA272372	ESTs, Weakly similar to match to ESTs AA316181 [H.sapiens]
no known gene	NO HUMAN CDNA		AA547022	ESTs, Weakly similar to TIA1_MOUSE NUCLEOLYSIN TIA-1 [M.musculus]
no known gene	NM_032947	AF313413	AA239554	ESTs
no known gene	NM_015696	AK027683	W35981	ESTs, Moderately similar to GLUTATHIONE PEROXIDASE [Schistosoma mansoni]
no known gene	NM_030782	BC025305	AA020034	ESTs, Weakly similar to cleft lip and palate transmembrane protein 1 [H.sapiens]
no known gene	NO HUMAN CDNA		AI530458	ESTs, Moderately similar to unnamed protein product [H.sapiens]
no known gene	NM_015242	AY049732	AA268881	ESTs, Highly similar to KIAA0782 protein [H.sapiens]
no known gene	NO HUMAN CDNA		AA030366	ESTs
sorting nexin 10	NM_013322	BC031050, BC147978	AA260397	ESTs, Weakly similar to SDP8 [M.musculus]
FLJ13433	NM_022496	AK023495	AA184337	ESTs, Weakly similar to ACTZ_HUMAN ALPHA-CENTRACTIN [M.musculus]

Mouse gene name	Human mRNA	Genbank Source	Genbank	Description
no known gene	no homologene		AA537509.1	Public domain EST {IMAGE:949810}
no known gene	no homologene		AA388607	ESTs
no known gene	no homologene		W09604	ESTs, Highly similar to large I antigen-forming beta-1,6-N-acetylglucosaminyltransferase [M.musculus]
no known gene	no homologene		W83671	ESTs, Weakly similar to proteolipid protein 2 [M.musculus]
major vault protein	NM_005115, NM_017458	AJ238510, AJ23519,X79882	AA200827	ESTs, Moderately similar to I53908 major vault protein - rat [R.norvegicus]
no known gene	no homologene		W08116	ESTs, Moderately similar to WDNM1 PROTEIN [Rattus norvegicus]
no known gene	no homologene		AA204090	ESTs, Weakly similar to AF201951_1 high affinity immunoglobulin epsilon receptor beta subunit [H.sapiens]
no known gene	no homologene		AA098237	ESTs
MDS006: X006 protein	NM_020233	BC001294	AA138584	ESTs
VMP1:likely ortholog of rat vacuole membrane protein 2	NM_030938	AF14006	AA516913	ESTs, Weakly similar to CG1534 gene product [D.melanogaster]
Hepcidin antimicrobial peptide	NM_021175	AJ277280	W12913	ESTs, Moderately similar to HEPC_HUMAN ANTIMICROBIAL PEPTIDE HEPCIDIN PRECURSOR [H.sapiens]
transmembrane 7 superfamily member 1	NM_003272	AF027826	AA189999	DNA segment, Chr 13, Abbott 1 expressed
no known gene	no homologene		AA122848	ESTs
mitogen-activated protein kinase kinase kinase 7 interacting protein 2	NM_145342, NM_015093	AB018276, AL117407	W82121	ESTs, Weakly similar to scaffold attachment factor B [R.norvegicus]
no known gene	no homologene		AA261222	ESTs
glycine amidinotransferase	NM_001482	S68805	AA185055	ESTs, Highly similar to GATM_RAT GLYCINE AMIDINOTRANSFERASE PRECURSOR [R.norvegicus]
phosphoinositide-3-kinase	NM_014308	AF128881	AA290057	ESTs
no known gene	no homologene		AA210357	ESTs
FLJ20401	NM_017805	AK000408	AI391280	ESTs, Highly similar to unnamed protein product [H.sapiens]
no known gene	no homologene		AA178549	ESTs
no known gene	no homologene		W11587	ESTs, Moderately similar to SARL_HUMAN SARCOLIPIN [H.sapiens]

Mouse gene name	Human mRNA	Genbank Source	Genbank	Description
no known gene	no homologene		AA163875	ESTs
no known gene	no homologene		AI595493	ESTs, Weakly similar to AF161080_1 inhibitory receptor PILRalpha [H.sapiens]
KIAA0475	NM_014864	AB007944	AA210038	ESTs
no known gene	no homologene		AA268055	ESTs
FLJ22833	NM_022837	AK026486	AA175979	ESTs, Weakly similar to CG5181 gene product [D.melanogaster]
placenta-specific 8	NM_016619	AF208846	AA245029	DNA segment, Chr 5, Wayne State University 111, expressed
Z39IG: Ig superfamily protein	NM_007268	AJ32502	AA261076.1	Public domain EST {IMAGE:720457}

Cluster D

Mouse gene name	Human mRNA	Genbank Source	Genbank	Description
no known gene	NM_05133	AE006639	W14214	ESTs
procollagen, type V, alpha 2	NM_000393	BC015705, M58529,Y14690	AA138290	ESTs
solute carrier family 29, member1	NM_004955	U81375	AI451844	ESTs, Highly similar to AF131212_1 equilibrative nitrobenzylthioinosine-sensitive nucleoside transporter ENT1 [M.musculus]
no known gene	NO HUMAN CDNA		W99891	ESTs
solute carrier family 29, member1	NM_004955	U81375	AA397253	ESTs, Highly similar to AF131212_1 equilibrative nitrobenzylthioinosine-sensitive nucleoside transporter ENT1 [M.musculus]
no known gene	NO HUMAN CDNA		W29300.1	Public domain EST {IMAGE:337567}
no known gene	NM_001908	AK092070	W41810	ESTs, Weakly similar to T17344 hypothetical protein DKFZp586L2024.1 - human [H.sapiens]
twisted gastulation protein	NM_020648	BC020490	AA267373	ESTs
adenosine deaminase RNA specific B1	NM_001112	U76420	W16053	ESTs
no known gene	NM_015429	AB056106	AA267567	ESTs
oxoglutarate dehydrogenase	NM_002541	D10523	W13320	ESTs, Highly similar to 2-OXOGLUTARATE DEHYDROGENASE E1 COMPONENT PRECURSOR [Homo sapiens]
no known gene	NM_016308	AF070416	AI594925	ESTs, Highly similar to URIDYLATE KINASE [Saccharomyces cerevisiae]
Mrps18b	NM_041046	AF100761	AI426268	ESTs, Moderately similar to PTD017 [H.sapiens]
no known gene	NO HUMAN CDNA		AA185432	ESTs
no known gene	NO HUMAN CDNA		AA461746	ESTs

Cluster E

Mouse gene name	Human mRNA	Genbank Source	Genbank	Description
no known gene	AA146022, AK026169		AA146022	ESTs
no known gene	NM_003505	AB017363	AI604159	ESTs
no known gene	NO HUMAN CDNA		W14925	ESTs, Moderately similar to KIAA1029 protein [H.sapiens]
no known gene	NM_014864	AB007944	AA274981	ESTs
no known gene	NO HUMAN CDNA		AA033308	ESTs
N4wbp-4 pending	NM_020182	AF305616	AA144094	ESTs, Highly similar to dJ718J7.1 [H.sapiens]
filamin like protein	AI678681		AA466198	ESTs, Highly similar to ENDOTHELIAL ACTIN-BINDING PROTEIN [Homo sapiens]
no known gene	NM_004518	Y15065	W11395	ESTs
secreted modular binding protein 2	NM_022138	AB014737	AA272826	ESTs, Weakly similar to AF070470_1 SPARC-related protein [M.musculus]
no known gene	NM_007080	AJ238098	W09867	ESTs, Moderately similar to HYPOTHETICAL 9.3 KD PROTEIN ZK652.1 IN CHROMOSOME III [Caenorhabditis elegans]
no known gene	NO HUMAN CDNA		AA274099	ESTs, Weakly similar to ZIP-kinase [M.musculus]
no known gene	NM_017510	BC001123	AA517431	ESTs, Moderately similar to GLYCOPROTEIN 25L PRECURSOR [Canis familiaris]
no known gene	AL832340, AL833405		AA386758	ESTs
no known gene	NO HUMAN CDNA		AA217009	ESTs
transforming growth factor beta 1 induced transcript 4	NM_006022	AJ222700	AA060863.1	Public domain EST {IMAGE:482995}
no known gene	NM_004265	AF084559	AA068575	ESTs, Weakly similar to delta-6 fatty acid desaturase [M.musculus]
cathepsin Z	NM_001336	AF136273	W14289	DNA segment, Chr 2, Wayne State University 143, expressed
janus kinase 1	NM_002227	M64174	W29699	ESTs, Highly similar to TYROSINE-PROTEIN KINASE JAK1 [Homo sapiens]
no known gene	NO HUMAN CDNA		W82178	DNA segment, Chr 7, Wayne State University 86, expressed
no known gene	NM_032849	AK055635	AA024250	ESTs
no known gene	NO HUMAN CDNA		AA002801.1	Public domain EST {IMAGE:426240}
no known gene	NM_001478	L76079, M83651	AA268669	ESTs, Weakly similar to AF232669_1 Kalirin-12a [R.norvegicus]
ATP binding cassette, subfamily A (ABC1), member 1	NM_005502	AF165281, AF275948	AA203809	ESTs
no known gene	NO HUMAN CDNA		W36470	ESTs, Weakly similar to T00343 hypothetical protein KIAA0584 - human [H.sapiens]
no known gene	NO HUMAN CDNA		AA050516	DNA segment, Chr 9, Wayne State University 18, expressed
no known gene	NO HUMAN CDNA		AI426270	ESTs, Weakly similar to nuclear protein np95 [M.musculus]

Mouse gene name	Human mRNA	Genbank Source	Genbank	Description
no known gene	NM_002358	U31278	AA466530	ESTs, Moderately similar to KIAA0280 [H.sapiens]
no known gene	NM_022763	AK027052	AA265864	ESTs
golgi SNAP receptor complex member 2	NM_004287, NM_054022	AF007548, AF229796	AA002301	ESTs
no known gene	NM_003387	AF031588, AF106062	AA174503	ESTs
lectin, mannose-binding,1	NM_005570	U09716, X71661	AA036111	ESTs, Highly similar to ERGIC-53 PROTEIN PRECURSOR [Homo sapiens]
ribosome binding protein 1	NM_004587	AF006751	AA002385	ESTs, Moderately similar to KIAA1398 protein [H.sapiens]
no known gene	NO HUMAN CDNA		AA139063	ESTs, Moderately similar to KIAA0007 [H.sapiens]
no known gene	NO HUMAN CDNA		AA189695	ESTs, Highly similar to TROPOMYOSIN 4, EMBRYONIC FIBROBLAST ISOFORM [Rattus norvegicus]
no known gene	NO HUMAN CDNA		AI449320	ESTs
quiescin Q6	NM_002826	U97276	AA024091	ESTs, Moderately similar to quiescin [H.sapiens]
no known gene	NM_019026	AB020980	AA472933	ESTs, Highly similar to unknown [H.sapiens]
no known gene	NO HUMAN CDNA		AA048837	ESTs, Highly similar to INTERFERON-INDUCIBLE PROTEIN [Rattus norvegicus]
no known gene	NO HUMAN CDNA		AA239252	ESTs
golgi reassembly stacking protein 2	NO HUMAN CDNA		AA213185	ESTs, Weakly similar to AF218940_1 formin-2 [M.musculus]
no known gene	NM_020123	AF160213	AA144167	ESTs, Highly similar to unnamed protein product [H.sapiens]
neuropilin 2	NM_003872	AF022860	AA269699	ESTs
no known gene	NO HUMAN CDNA		AA217196	ESTs
no known gene	NO HUMAN CDNA		AA432472.1	Public domain EST {IMAGE:833346}
calreticulin	NO HUMAN CDNA		W33774.1	Public domain EST {IMAGE:352406}
no known gene	NO HUMAN CDNA		AA177584.1	Public domain EST {IMAGE:621742}
no known gene	NM_020820	AJ320261	W82294	ESTs
no known gene	NM_018446	AF157318	AA210344	ESTs, Highly similar to AF157318_1 AD-017 protein [H.sapiens]
lectin, mannose-binding,1	NM_005570	U09716, X71661	AA244713	ESTs, Highly similar to ERGIC-53 PROTEIN PRECURSOR [Homo sapiens]
no known gene	NO HUMAN CDNA		AA437983	ESTs, Weakly similar to AF151373_1 nucleolin-related protein NRP [R.norvegicus]
no known gene	NM_001643	BC007309	AA404092	ESTs, Moderately similar to COATOMER DELTA SUBUNIT [Homo sapiens]
tripartite motif protein 8	NM_030912	AF281046	AA027381	ESTs, Weakly similar to I49642 estrogen-responsive finger protein - mouse [M.musculus]
no known gene	NM_014604	AF028823	AI893697	ESTs, Highly similar to HYPOTHETICAL 13.5 KD PROTEIN C45G9.7 IN CHROMOSOME III [Caenorhabditis elegans]

Mouse gene name	Human mRNA	Genbank Source	Genbank	Description
no known gene	NO HUMAN CDNA		AA265636	ESTs, Highly similar to CALDESMON, SMOOTH MUSCLE [Gallus gallus]
no known gene	NO HUMAN CDNA		AA536838	ESTs
filamin like protein	AI678681		AA003323	ESTs, Highly similar to ENDOTHELIAL ACTIN-BINDING PROTEIN [Homo sapiens]
no known gene	NM_020790	AF201945	W14353	ESTs, Weakly similar to trabecular meshwork-induced glucocorticoid response protein [M.musculus]
no known gene	NO HUMAN CDNA		AA260155	DNA segment, Chr 2, Wayne State University 127, expressed
platelet-derived growth factor receptor-like	NM_006207	D37965	AA030377	ESTs, Highly similar to PDGF receptor beta-like tumor suppressor [H.sapiens]
no known gene	NM_014933	AB018358	AA544844	ESTs, Moderately similar to T14150 vesicle associated protein 1 - rat [R.norvegicus]
no known gene	NO HUMAN CDNA		W10776.1	Public domain EST {IMAGE:314509}
no known gene	NM_032849	AK055635	AI552496	ESTs
no known gene	NM_004394	X76105	AA269524	ESTs, Highly similar to DAP1_HUMAN DEATH-ASSOCIATED PROTEIN 1 [H.sapiens]
no known gene	NO HUMAN CDNA		W97172	DNA segment, Chr 13, Wayne State University 115, expressed
no known gene	NM_012426	D87686	AA269584	ESTs, Highly similar to KIAA0017 protein [H.sapiens]
enolase 1, alpha non neuron	NM_001428	X16287	AA204262	ESTs, Highly similar to ALPHA ENOLASE [Mus musculus]
no known gene	NO HUMAN CDNA		AA172597	ESTs
no known gene	NO HUMAN CDNA		AA237920	ESTs

Table 3: Characteristics of Clusters A through E

Cluster	Early	Late
A	↓	↓
B	-	↑
C	↑	-
D	↑	↑↑
E	↑	↑

↓= gene expressed reduced at least 2 fold. ↑ = gene expression increased at least 2 fold. ↑↑ = gene expression increased more than 2 fold.

Example 3

Confirmation of microarray data by RT-PCR and In situ Hybridization

[0072] Confirmation of the microarray data was performed by measuring the expression level of genes in two individual paws at each time point using real time RT-PCR and *in situ* hybridization.

[0073] Real time reverse transcription (RT) PCR analysis was performed as follows: to remove possible genomic DNA contamination, total paw RNA was treated with amplification grade DNAse I (Gibco Life Technologies, Rockville, MD). RNA was then subjected to reverse transcription using SUPERSCRIPT Preamplification System for First Strand cDNA Synthesis (Gibco Life Technologies). Serial dilutions of the cDNA template were prepared and PCR was carried out using a Lightcycler System (Roche Molecular Biochemicals, Palo Alto, CA). After each elongation phase, the fluorescence of SYBR Green I, which binds double-stranded DNA was measured. Reactions (20 μl) were performed in microcapillary tubes using 5 μl of diluted cDNA with SYBR Green I (Roche Molecular Biochemicals), master mix, upstream and downstream primers and $MgCl_2$. Sequences of primer pairs were as follows: Follistatin-like, upstream: 5'-GGA TTG AGA ATC AGC ACT GGG-3' (SEQ ID NO:386); downstream: 5'-TTG AAA GGG AGG GCA CAG AAC-3' (SEQ ID NO:387); IL-2Rα, upstream: 5'-CGG AAG CCT GAA CAT CAA TCC-3' (SEQ ID NO:388); downstream: 5'-GCC ACT AAC CCC AAC TCT TAT GAG-3' (SEQ ID NO:389); GAPDH, upstream: 5'-ACC ACA GTC CAT GCC ATC AC-3' (SEQ ID NO:390); downstream: 5'-TCC ACC ACC CTG TTG CTG TA-3' (SEQ ID NO:391). Reactions containing water or cDNA synthesized without reverse transcriptase, as template, resulted in no PCR products. Dilutions of cDNA synthesized from early paw RNA were predicted to have the highest expression of the gene product being amplified and, thus, were used as the concentration standards. Lightcycler quantification software v3 was used to compare amplification in experimental samples during the log-linear phase to the standard curve from the dilution series of acute tissue. All experimental samples were normalized to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) expression levels for that tissue. Expression levels of each gene were plotted relative to the levels in normal tissue.

[0074] *In situ* hybridization analysis was performed as previously described (Witte, et al. Am J. Pathol 1991;139:717-724). Briefly, ten micron cryostat sections of snap frozen tissue were air dried on TESPA coated Superfrost Plus (Histology Control Systems, Glenhead, New York) slides and post-fixed in 4% (w/v) paraformaldehyde in PBS then acetylated with acetic anhydride as described. Paws were fixed for 48 hours in 4% (w/v) paraformaldehyde (Electron Microscopy Sciences, Ft. Washington, PA) in PBS at 4°C immediately after harvesting. Following fixation, the tissue was decalcified in TBD-2 (Shandon, Pittsburgh, PA). Complete decalcification of the tissue was determined using 5% ammonium oxalate. Following decalcification the tissue was rinsed for ten minutes in running water and placed in 30% sucrose in PBS for 24 hours at 4°C. The samples were embedded in M-1 mounting media (Shandon), frozen in liquid nitrogen and stored at -80°C. Hybridizations were done overnight at 45°C under a sealed coverslip. Following hybridization, the sections were treated with RNAse to remove unbound probe and the slides were washed extensively under highly stringent conditions. The slides were developed in Kodak D19 developer (Rochester, NY). Sections were counterstained with hematoxylin & eosin and photographed using both dark- and bright-field illumination.

[0075] Mouse sense and antisense RNA probes were synthesized using the RNA Transcription Kit (Stratagene, La Jolla, CA). T3 or T7 RNA polymerase produced ^{35}S- radiolabeled antisense or sense single-stranded RNA probes, respectively. A sense probe generated from an unrelated mouse gene was used as a negative control for *in situ* hybridization.

[0076] Although none of the genes previously demonstrated to be upregulated by RPA were present on the microarray chip, two genes on the DNA microarray were related to genes whose expression patterns we have previously analyzed by RPA. One of the genes, IL-2Rγ, had a similar expression pattern to the previously observed expression pattern of IL-2. Another gene, follistatin-like, which is induced by TGFβ, had a similar expression pattern to the previously observed expression patterns of TGFβ 1, 2 and 3. Comparison of the expression of follistatin-like and IL-2Rγ by microarray and real time RT-PCR revealed similar patterns of expression (Figure 2). In addition, spatial expression of IL-2Rγ was analyzed by *in situ* hybridization (Figure 3). The expression pattern matched that observed in the DNA microarray hybridizations. IL-2Rγ was expressed in the inflammatory tissue surrounding the joint and in the periosteal tissue along the length of the bone.

Example 4

Classification of differentially expressed genes

[0077] Of the 385 genes that were found to be differentially expressed during CIA in the mouse paw, 102 were expressed sequence tags (ESTs) and preferred members of this group represent novel genes critical to the pathology of CIA. Excluding duplicate gene spotting on the

chip, 240 of the 385 gene sequences are annotated genes. Information on their expression in various tissues was obtained using LocusLink and Unigene at the National Center for Biotechnology Information (NCBI) website (ncbi.nlm.nih.gov/). These genes have been reported in a variety of tissues, including but not limited to bone, brain, colon, liver, lung, kidney, mammary, skin, spleen and testis. Not surprisingly, the majority are expressed in the lymphoid organs, including spleen and lymph nodes (Figure 4).

[0078] To further characterize the annotated genes, they were grouped into categories using Incyte's Function and Pathways categorization (Figure 5). The largest functional categories included immunity and defense (47 genes), protein metabolism (36 genes), lipid metabolism (11 genes) and differentiation and proliferation (11 genes). The largest pathways categories included membrane (59 genes), secreted and extracellular (59 genes), organelle (24 genes), intracellular signaling (17 genes), receptors (17 genes), proteases (15 genes) and antigen recognition (14 genes). In most cases, the genes in each category were distributed proportionally to the size of the clusters identified in Figure 1.

[0079] The 240 previously characterized genes that were differentially regulated during CIA were analyzed through extensive literature searches. Of these 240 genes, a number of genes that have not previously been characterized in autoimmune arthritis but that could potentially be involved, were identified. From the literature searches on these particular genes, a number of genes were found to be associated with three basic biological functions. These genes, as well as their temporal expression, are listed in Table 4.

Table 4 Genes novel to arthritis

	Early	Late
Proliferation, differentiation and tumorigenesis		
enolase 3, β muscle		*
tumor-associated calcium signal transducer 2		*
S100 calcium binding protein A3		*
angiopoietin related protein 2		*
β-1,4 N acetylgalactosaminyltransferase	*	*
polypeptide N-acetylgalactosaminyltransferase 1	*	*
endomucin	*	*
growth factor receptor bound protein 10	*	*
growth arrest and DNA-damage-inducible, γ	*	*
dickkopf homolog 3 (Xenopus laevis)	*	*
CDC28 protein kinase 1	*	
a disintegrin and metalloproteinase domain 9	*	
ecotropic viral integration site 2	*	
selenoprotein P	*	
proprotein convertase subtilisin/kexin type 5	*	
B-cell leukemia/lymphoma 3	*	

Apoptosis		
apoptotic protease activating factor 1	*	*
regulator of G-protein signaling 5	*	*
calumenin	*	*
CD97	*	*
calpain 6	*	*
caspase 11	*	*
receptor interacting protein	*	*
transglutaminase 2, C polypeptide	*	*
CD44	*	*
CD53	*	
fibrinogen/angiopoietin-related protein	*	
baculoviral IAP repeat-containing 2	*	
uncoupling protein 2, mitochondrial	*	

Inflammation		
annexin A2	*	*
annexin A4	*	*
annexin A6	*	*
lysosomal membrane glycoprotein 1	*	*
protocadherin 13	*	*
catenin beta	*	*
pentaxin related gene	*	
small proline-rich protein 2A	*	
small inducible cytokine subfamily B (Cys-X-Cys)	*	
colony stimulating factor 2 receptor, β 2, low-affinity	*	
CD37	*	
type II transmembrane protein	*	
BP-3 alloantigen	*	
Mus musculus hypoxia induced gene 2 (Hig2)	*	

[0080] The present study quantitatively analyzed coordinated gene expression on a global scale from paws of mice with CIA to identify novel genes involved in arthritis as well as to identify gene expression patterns that differ between early and late synovitis in this model system. Genes known to be upregulated in CIA or RA were confirmed by the analysis. However, most of the differentially-expressed genes identified by the microarray have not been previously described in arthritis.

[0081] The difference in expression profiles observed between early and late disease has not previously been fully-appreciated. Even though the microarray analysis was limited to two time points over the course of the disease, cluster analysis grouped the 385 genes according to their mRNA expression in early versus late disease. In some embodiments, the hierarchical clusters can represent coordinately expressed genes, the effects of cell phenotype and/or a combination of the two. Confirmation of the validity of the microarray expression analysis includes RT-PCR analysis of expression of follistatin-like gene and IL-2Rγ, as well as analysis of the spatial expression of IL-2Rγ by *in situ* hybridization. Of 385 genes on the microarray found to be differentially expressed in

CIA, 240 have been previously annotated. These 240 genes can be divided into several biological functions and pathways; however, none of the clusters were over-represented in any of these categories.

[0082] Included in the group of annotated genes are many that have previously been demonstrated to be upregulated in RA, including TIMP-3, β-2 microglobulin, biglycan, lumican, insulin-like growth factor binding protein 5 and stromal cell derived factor-1, as well as proinflammatory genes such as IL-2Rγ, small inducible cytokine A12 and A4 (MCP5 and MIP1β respectively), CCR5, macrophage expressed gene 1, cathepsins C and S, CD14 and fibronectin. Expression of a majority of these 240 genes also occurs in lymphoid organs, which is expected since the synovial inflammation is dominated by immune cells.

[0083] The 240 annotated genes were analyzed through extensive review of the literature, resulting in a list of 43 genes not previously characterized in autoimmune arthritis. Based on their known biological functions these genes might play central roles in the pathophysiology of the disease. These genes, as well as their temporal expression, are listed in Table 4. Several interesting comparisons can be made between the biological function of these genes, their temporal expression patterns, and the histopathologic appearance of arthritis.

Example 5

Genes expressed throughout CIA

[0084] Several genes involved in cell proliferation, differentiation and tumorigenesis were upregulated throughout the disease (clusters D and E). These included β-1,4 N-acetylgalactosaminyltransferase and polypeptide N-acetylgalactosaminyltransferase 1, that are involved in the synthesis of gangliosides, whose overexpression is associated with a marked increase in growth rate and invasive activity.

[0085] Numerous genes involved in apoptosis were identified that were expressed both in early and late disease. Cellular turnover in normal tissues is tightly regulated through a balance of cell proliferation and cell death. The regulation of cell populations within the joint is very likely also controlled by apoptotic processes. Apoptosis of cells within the arthritic joint has been proposed to be a source of self-peptides that could generate auto-antigens that may propagate inflammation. One of these, CD44, has been postulated to play a role in the elimination of neutrophils from sites of inflammation in inflammatory kidney disease and its upregulation on the surface of chondrocytes may contribute to cartilage degeneration in RA patients. Other genes include calpain 6 and caspase 11, which are members of two families of cysteine proteases involved in the regulation of pathological cell death. Additionally, receptor interacting protein (RIP) interacts with Fas, causing morphological changes in cells that resemble apoptosis.

[0086] Inflammatory processes occur both early and late in disease. Therefore, the identification of genes involved with inflammation was not unexpected; however, various genes were identified that had not previously been associated with inflammation in CIA or RA. These genes include annexins A2, A4 and A6, which affect the activation and migration of macrophages. The human homologue of lysosomal membrane glycoprotein 1, h-LAMP1, is detectable in patients with scleroderma and systemic lupus erythematosus and may contribute to the migration of activated leukocytes to the sites of inflammation. Catenin-β, when complexed with E-cadherin, is upregulated in gut inflammation of patients with spondyloarthropathy.

Example 6

Genes expressed in late CIA

[0087] Late CIA is characterized by an increase in fibrosis. Fibroblasts taken from RA patients with chronic disease are in a constitutive state of activation and exhibit plasticity in cell growth. Of the eight annotated genes that are selectively upregulated in late disease listed in cluster B of Table 1, four are involved in cell proliferation, differentiation and tumorigenesis and may play a role in the chronic activation of fibroblasts at late stages of disease. Specifically, tumor associated calcium signal transducer 2 is expressed early in tumorigenesis, and angiopoietin related protein 2 is associated with endothelial cell development and tumorigenesis.

Example 7

Genes expressed in early CIA

[0088] Several genes involved in cell proliferation, differentiation and tumorigenesis are selectively upregulated in early disease and are listed in cluster C of Table 1. CDC28 kinase binds to the catalytic subunit of cyclin dependent kinases and may be associated with dysregulation of lymphocyte cell cycle control in HIV infected patients. ADAM9, a disintegrin and metalloproteinase domain 9, binds MAD2beta, which is involved in cell cycle control.

[0089] Three apoptosis genes that are selectively upregulated in early CIA have anti-apoptotic properties. These include CD53, fibrinogen/angiopoietin related protein and baculoviral IAP repeat containing 2. The latter two are involved in endothelial cell survival. The upregulation of genes involved in endothelial cell survival, particularly early in disease, may allow for migration of inflammatory cells into the diseased joint.

[0090] Genes selectively upregulated in early arthritis (cluster C) include many inflammatory genes previously associated with CIA or RA. In addition, numerous other potentially pro-inflammatory genes are in this category. Pentaxin-related gene is involved in inflammatory reactions, particularly those of the vessel wall. Small inducible cytokine B subfamily member 13 (CXCL13) is a chemokine for B lymphocytes. Type II transmembrane protein is expressed exclusively in macrophages and monocytes and is involved in activation of myeloid cells. Hypoxia

induced gene 2 (interleukin-20) is modulated by hypoxia and may have a role in inflammation, possibly in attempting to re-establish homeostasis.

Example 8

Genes that are down-regulated

[0091] Although most of the differentially-expressed genes were upregulated during CIA, all the genes in cluster A of Table 1 were downregulated, compared to normal paws. This represents a group of potentially important genes, as their downregulation may contribute to the loss of homeostasis in the joint and the failure to limit the inflammatory process. One annotated gene in cluster A, cytochrome P450, has previously been shown to be downregulated in inflammation and certain alleles of cytochrome P450, which are inactive or poor metabolizers, show a modest association with susceptibility to ankylosing spondylitis, but not RA. Most of the genes in cluster A are ESTs, and their further characterization will be of interest. In addition to the 25 ESTs in cluster A, the further characterization of the other 132 ESTs identified in this study will provide information about the gene regulatory network(s) involved in the autoimmune arthritic process.

[0092] In summary, the present study utilized DNA microarray technology to analyze coordinated gene expression in paws of mice with early and late CIA. This analysis has revealed a large number of genes previously not known to be involved in arthritis, as well as distinct gene expression profiles that differentiate between early and late CIA. Further characterization of these genes and pathways will advance the understanding of the basic mechanisms responsible for initiation and persistence of synovitis and may aid in the development of novel therapies.

Example 9

Isolation of full-length genes identified by ESTs

[0093] The 157 expressed sequence tags (ESTs) are used to identify the full-length genes associated with them. The EST sequences are used to search public and proprietary computer databases. Those that are not identified in the databases, are used to screen mouse libraries for full-length cDNA clones using methods known to one of skill in the art.

Example 10

Identification of Human Homologs and production of a human microarray

[0094] Human homologs are identified by searching databases to find the closest human homolog for each of the 385 mouse genes identified herein. Many of the human homologs are known. Those that do not possess a homolog in the databases are identified by screening a human cDNA library using a mouse probe. In particular, when active regions or highly conserved regions of the mouse protein are known, these are used to screen the library. For example, kinases

are known to contain regions that are highly conserved. Thus, if the mouse gene codes for a kinase, these regions are included within the probe. Alternatively, or in addition, a degenerate mouse probe is produced, with the degeneracy in regions that are less likely to possess high homology, for example, a degenerate probe for a kinase is constructed to have more degeneracy around the kinase region.

Example 11

mRNA expression profiling of early and late rheumatoid arthritis in humans

[0095] Differential gene expression in the synovial tissue of humans with rheumatoid arthritis was analyzed and compared to that of synovial tissue from normal humans.

[0096] RNA was isolated from a human synovial biopsy and quick frozen in liquid nitrogen for storage at -80°C. Frozen synovial tissue was minced with a scalpel and homogenized with a Polytron Tissue Tearor (Biospec Products, Bartlesville, OK) in appropriate volumes of RNA Stat-60 (Tel-Test, Friendswood, TX). Total RNA was extracted from the tissue homogenates according to the manufacturer's instructions. Pooled total RNA from normal synovial biopsy samples, mild arthritic synovial biopsy samples and severe arthritic synovial biopsy samples was used to isolate polyA+ RNA using the Oligotex mRNA isolation kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. RNA concentrations were measured by fluorometry using the Ribogreen RNA Quantification Kit (Molecular Probes, Inc., Eugene, OR).

[0097] DNA microarray analysis was performed as follows: mRNA from a human without RA was used for normalization of gene expression levels across all microarray chips. Competitive hybridizations with Cy3 labeled normal human mRNA versus Cy5 labeled mild RA mRNA or Cy5 labeled severe RA mRNA were performed. Each sample (normal, mild and severe) was labeled and hybridized to the GeneChip® Human Genome U95 Set from Affymetrix (Santa Clara, CA)which represents about 60,000 full-length genes and EST clusters.

[0098] Primary data is examined using Incyte Gemtools software and GeneSpring version 4.0.4 software (Silicon Genetics, Redwood City, CA). Defective cDNA spots (irregular geometry, scratched, or <40% area compared to average) or spot fluorescence hybridizations with signal to noise ratios less than 2.5:1 are eliminated from the data set. Data sets are subjected to normalization first within each microarray experiment such that the median of the Cy5 channel was balanced against the ratio of the Cy3 channel (k*(MedianCy3) = MedianCy5, where k is the ratio of the median intensities in each). Each microarray contained 192 control genes present as non-mammalian single gene "spikes" or "complex targets". The complex targets consist of probe-sets that contain a pool of cellular genes expressed in most cell types. In addition, each experimental mRNA sample was augmented with incremental amounts of non-mammalian gene RNA (2X, 4X, 16X, etc) to permit assessment of the dynamic range attained within each microarray. Little variation was observed across the microarray series with respect to the control genes (not shown),

providing support for inter-array comparisons of temporally regulated genes. Genes were clustered according to their expression pattern by subjecting the log-transformed data (R= $\log_2$Cy5/(kCy3), where R is the log of the expression ratio for each gene) to the hierarchical tree clustering algorithm as implemented in the GeneSpring program (Silicon Genetics). The hierarchical tree analysis was performed using a minimum distance value of 0.001, separation ratio of 0.5 and the standard correlation distance definition.

[0099] Human sense and antisense RNA probes were synthesized using the RNA Transcription Kit (Stratagene, La Jolla, CA). T3 or T7 RNA polymerase produced ^{35}S- radiolabeled antisense or sense single-stranded RNA probes, respectively. A sense probe generated from an unrelated human gene was used as a negative control for *in situ* hybridization.

[0100] For mild and severe disease, mRNA from patients with severe arthritis (score of 4) were used to generate probes that are hybridized to the GeneChip® Human Genome U95 Set from Affymetrix (Santa Clara, CA) which represents about 60,000 full-length genes and EST clusters, as is mRNA from normal human synovial tissue. Hybridizations are conducted on duplicate chips, allowing for the elimination of genes whose expression levels differed by greater than 50% between the duplicate samples. About 60,000 genes and ESTs are represented in the Set.

[0101] The method above seeks to identify all genes that are differentially expressed in human arthritis using a variety of microarrays or DNA chips. Using the information identified in Examples 9-11 a "human Rheumatoid Arthritis genechip" is produced.

Example 12

Method for the production of a "human Rheumatoid Arthritis genechip"

[0102] The genes that are found to be differentially expressed in Examples 9-11 are used to produce a "human Rheumatoid Arthritis genechip." This chip will be used for the diagnosis, prognosis, and treatment of the disease.

[0103] Other chips are produced with those differentially expressed genes that are only expressed in mild disease, a "mild RA" chip and those that are only differentially expressed in severe disease, a "severe RA" chip.

Example 13

Method for the diagnosis and staging of RA

[0104] mRNA is isolated from human synovial tissue, blood and human synovial fluid and treated as in Example 2. The microarray produced in Example 12 is analyzed for gene expression. From the analysis of up-and down-regulated genes a diagnosis and analysis of disease is made. The patient is monitored periodically during active disease and/or treatment. A prognosis is made based on these results as to the severity and chronic nature of the disease as well as the speed of deformity.

Example 14

Treatment of RA by inhibiting expression of up-regulated genes

[0105] One or more of the genes that are up-regulated in Examples 4-6 are inhibited using antisense oligonucleotides or triple helix oligonucleotides. The antisense oligonucleotides are produced using methods known to one of skill in the art. The antisense oligonucleotides are administered intravenously, intramuscularly, or within a joint and the symptoms and disease is monitored.

Example 15

Treatment of RA by activating expression of down-regulated genes

[0106] One or more of the genes that are down-regulated in Example 7 are activated using known transcriptional activators. Alternatively, expression vectors are administered that are targeted to the synovia and express one or more of the genes that are down-regulated. Preferably, the expression vectors are retroviral and are administered intravenously. The transcriptional activators and vectors are produced using methods known to one of skill in the art.

Example 16

Treatment of RA by administration of down-regulated proteins

[0107] One or more of the proteins that are down-regulated in Example 7 are purified and administered. The proteins are administered intravenously or into the joint.

Example 17

Use of fibrinogen/angiopoietin-related protein to enhance angiogenesis in synovial tissues and to define the involvement in arthritic processes

[0108] Because primers for fibrinogen/angiopoietin-related protein amplified a 270 base pair product from cDNA synthesized from mRNA from synovial tissues of RA patients, this suggests that this protein is involved in some way in the pathogenic process. Thus, expression of fibrinogen/angiopoietin-related protein is analyzed in various forms of RA and in situ in synovial tissue. If over-expression is identified in the process, anti-sense oligonucleotides are used to inhibit expression of fibrinogen/angiopoietin-related protein in synovia or systemically in the RA patients.

Example 18

Determination of the best treatment for a patient with RA

[0109] From the results of the gene expression analysis, the best treatment for the patients with RA is determined. The treatment is based on the specific gene expression profile.

[0110] Thus, synovial fluid from a patient with rheumatoid arthritis is analyzed using a microarray as in Example 2. The analysis is used to identify the genes that are specifically up-

regulated or down-regulated in that patient. Then, the treatment is selected based on the specific gene expression.

[0111] Although described in the context of certain preferred embodiments, the skilled artisan will appreciate that various changes and modifications can be made to the preferred embodiments, and such changes and modifications are meant to be encompassed by the invention, as defined by the appended claims.

Example 19

Correlation of mRNA overexpression in CIA with human gene and function: FARP

[0112] Microarray analyses identified fibrinogen/angiopoietin related protein (FARP) as one of the most highly over-expressed mRNAs (8734 tested) in arthritic paws of mice with collagen-induced arthritis (CIA). *See* Table 1, Cluster C, Mouse # W13905; Fibrinogen/angiopoietin-related protein. Data also demonstrated that human FARP. Data also demonstrated that human FARP mRNA is expressed in rheumatoid arthritis (RA) synovium. FARP is highly homologous to angiogenic factors and inhibits apoptosis of vascular endothelial cells *in vitro*. In RA, an increase in blood vessel formation, or angiogenesis, is observed in synovial tissue. Endothelial cells lining blood vessels can provide nutrients for inflamed tissue, allow infiltration of inflammatory cells, and secrete inflammatory cytokines, all of which contribute to disease processes. The suppression of arthritis by angiogenic inhibitors in animal models, such as CIA, further demonstrates that angiogenesis is necessary for arthritis. Mouse FARP mRNA is highly expressed during early stages of CIA and human FARP mRNA is expressed in RA synovial tissue.

Example 20

Characterizing FARP expression in CIA

[0113] Prior to the present invention, FARP had not been described in arthritis. Localization of the cells that produce FARP mRNA and protein within the joint permits analysis FARP's role in angiogenesis in CIA. The cell types producing FARP mRNA and protein are determined and the role of FARP protein expression as it relates to the mRNA expression during CIA is identified.

[0114] **Determination of spatial expression of FARP mRNA during CIA.** DBA/1 mice are immunized with collagen as described in Thornton, et al. (1999) *Arthritis Rheum* 42:1109-1118. Mice are sacrificed 21, 28, 35, 42 and 49 days following primary collagen immunization. *In situ* hybridization analysis of FARP mRNA expression using sense and antisense probes generated from the FARP mouse cDNA are performed on tissue sections from paws of normal, unimmunized mice and arthritic mice.

[0115] **Generation of antibody to FARP.** An anti-FARP antibody is generated as described in Kim I, et al, (2000) *Biochem J* 346:603-610, and used for immunodetection and

blocking of FARP function. Nucleotides 298 to 866 of the cDNA coding for the mouse FARP protein are cloned into the mammalian expression vector pcDNA3.1/His, which incorporates a histidine tag for easy isolation of the recombinant protein (Invitrogen, Carlsbad, CA). Following purification, this protein fragment encoding amino acids 100-289 of mouse FARP is injected into rabbits and serum is collected. Polyclonal antibody is purified from rabbit serum by ammonium sulfate precipitation and protein A column chromatography as described in Harlow E, et al, (1988) *Antibodies: A laboratory manual*. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory; and Shanley JD, et al, (1994) *J Infect Dis* 169:1088-1091.

[0116] **Determination of spatial and temporal expression of FARP protein during CIA.** Since protein levels do not always directly reflect mRNA levels of a gene, the protein expression of FARP is determined in arthritic CIA paws using the anti-mouse FARP polyclonal antibody generated above. FARP protein is localized immunohistochemically using a horseradish peroxidase conjugated anti-rabbit secondary antibody. Sections are processed from paws of non-immunized mice and from paws of mice sacrificed 21, 28, 35, 42 and 49 days following primary collagen injection. Sera from non-immunized rabbits are used as a negative control. Sections from mouse liver are used as a positive control for immunohistochemical staining.

[0117] **Results.** *In situ* mRNA analysis demonstrates expression of FARP mRNA in the inflamed area of arthritic paws. FARP mRNA and protein are seen to be more highly expressed early in disease. In some embodiments, FARP protein is localized to the vasculature in arthritic paws. Blood vessel formation in CIA paws is readily observed by standard hematoxylin and eosin staining. However, co-localization of vasculature and FARP expression is demonstrated by analysis of serial sections for expression of endothelial cell-specific markers, such as von Willebrand factor Lu J, et al, (2000) *J Immunol* 164:5922-5927, in conjunction with FARP expression. The anti-human FARP polyclonal Ab from Kim, et. al. will be obtained, as this antibody will likely crossreact with mouse FARP. The homologous portion of mouse FARP protein that was previously used by Kim, et. al., is used to generate anti-human FARP polyclonal antibodies. Successful use of this polyclonal antibody in immunohistochemical staining demonstrates that administration of this portion of the protein to rabbits can generate polyclonal antibody to FARP. Polyclonal antibodies are easier and faster to generate than monoclonal antibodies; in some embodiments, the use of an antibody to block FARP function involves generation of a monoclonal antibody.

Example 21

Determining the anti-apoptotic effects of FARP on endothelial cells

[0118] The angiogenic protein Ang1 and FARP have anti-apoptotic effects on endothelial cells. Ang1 mediates its anti-apoptotic effects by activating Tie2, an endothelial cell-specific receptor, resulting in phosphorylation of the serine-threonine kinase, Akt (protein kinase B)

and mRNA upregulation of the apoptosis inhibitor, survivin. Papapetropoulos A, et al, (2000) *J Biol Chem* 275:9102-9105. FARP does not bind Tie2, but is highly homologous to Ang1 and is a secreted protein with anti-apoptotic effects on endothelial cells. FARP also has anti-apoptotic effects specific for endothelial cells, and is a secreted protein. Activation by FARP of an endothelial cell-specific receptor is found to result in the phosphorylation of specific anti-apoptotic intracellular molecules and increases mRNA expression of anti-apoptotic factors. Determination of the pathway that FARP utilizes in prolonging endothelial cell survival provides potential targets for therapeutic intervention. The effects of FARP on anti-apoptotic factors potentially regulating endothelial cell survival is identified.

[0119] In preferred embodiments, treatments and drug candidates that interfere with receptor binding by FARP lead to deactivation of the anti-apoptotic serine-threonine kinase, Akt, in endothelial cells. In further preferred embodiments, interference with expression of FARP, normal function of its receptor, and/or binding of FARP to its receptor also leads to decreased expression of survivin, Bcl2, and other anti-apoptotic factors in endothelial cells. Overall, these effects result in enhanced or normalized apoptosis of vascular endothelial cells in the arthritic joint, leading to a diminution or reversal of disease symptoms.

[0120] **Expression and purification of recombinant mouse FARP (rmFARP).** The entire cDNA coding for mouse FARP is inserted into the mammalian expression vector pcDNA3.1/His, which contains a six amino acid histidine tag for easy isolation of the protein (Invitrogen). The cDNA is transfected into COS-7 cells and purified from the cell supernatant. The anti-mouse FARP polyclonal antibody discussed above is used in Western blots to determine whether rmFARP is expressed in COS-7 cells.

[0121] **Effects of FARP on endothelial cell expression of anti-apoptotic molecules.** HUVEC (ATCC, Rockville, Maryland) is treated with rmFARP in a range of 50 to 500 ng/ml as described for Ang1 (Papapetropoulos A, et al, (2000) *J Biol Chem* 275:9102-9105) and or with vehicle. RNA from these cells is analyzed by RNase protection assays (BD Pharmingen, San Diego, CA) for expression of the anti-apoptotic genes, survivin and Bcl-2, as previously performed in Thornton S, et al, (1999) *Arthritis Rheum* 42:1109-1118.

[0122] **Effects of rmFARP administration on phosphorylation of serine-threonine kinases important in cell survival.** Phosphorylation of the Akt survival serine threonine kinase is assessed as described in Papapetropoulos A, et al. Microvascular endothelial cells (Vec Technologies, Rensselaer, NY) are treated with and without rmFARP. Anti-Akt antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and phosphospecific Akt antibody (New England Biolabs, Beverly, MA) are used in Western blots to determine the amount of Akt protein present and the extent of Akt phosphorylation in these cells.

[0123] **Results.** rmFARP is found to increase the expression of survivin or Bcl-2 in endothelial cells, and also increases the phosphorylation of Akt. FARP is found to utilize a separate

signaling pathway from Ang1, and other signaling molecules are thus analyzed for their role in the anti-apoptotic effects mediated by FARP. Additionally the anti-apoptotic molecules XIAP, c-IAP2 and NIAP are analyzed at the same time as survivin and Bcl-2 in the RNase protection analysis. These studies elucidate FARP's downstream effects that are mediated by a receptor.

Example 22

Determining the role of FARP during CIA

[0124] Since FARP is one of the most highly overexpressed genes in CIA, and since it is also expressed in rheumatoid arthritis synovial tissue, its role in arthritis is tested both by administration and depletion of FARP before disease onset and during disease progression. In some embodiments, FARP aids in endothelial cell survival, allowing for increased inflammation in CIA. Thus, treatment with FARP can exacerbate CIA, and depletion of FARP can inhibit CIA. Recombinant mouse FARP, as well as antibodies to FARP, are administered before and during disease.

[0125] **Effects of administration of rmFARP on the development and severity of CIA.** rmFARP is administered i.p. to DBA/1 mice immunized with collagen. Based on published studies with other molecules (Thornton S, et al, (2000) *J Immunol* 165:1557-1563), FARP (10 ug/0.5 ml/mouse) is administered twice daily from days 14 to 21 following primary collagen immunization for testing effects before disease onset. For established disease, FARP is administered twice daily for seven days starting 24 hours after disease onset. Mice are scored daily for macroscopic signs of arthritis as described in Thornton S, et al, (1999) *Arthritis Rheum* 42:1109-1118. Mice are sacrificed at day 49 of disease and sections from treated and untreated mouse paws are analyzed histochemically for blood vessel formation and inflammatory cell infiltration by hematoxylin and eosin staining.

[0126] **Effects of depletion of FARP on endothelial cell apoptosis.** Antibody produced as described herein is used. Assessment of the ability of anti-FARP antibody to block the anti-apoptotic effects of FARP is performed *in vitro* with endothelial cell lines as described in Kim I, et al, (2000) *Biochem J* 346 Pt 3:603-610. Induction of apoptosis in HUVEC cells is performed by serum deprivation. HUVEC cells are grown for 24 hours in the presence of 10% serum and then incubated for 24 hours with the same media, or serum-free media with control buffer, rmFARP (200 and 800 ng/ml) or rmFARP plus anti-FARP antibody at varying concentrations. Analysis of apoptotic cells is as described in Kim, et al. Sera from unimmunized rabbits is used as a negative control.

[0127] **Effects of depletion of FARP on the development and severity of CIA.** Anti-FARP antibody is administered similarly to studies using anti-VEGF antibody in CIA (Sone H, et al, (2001) *Biochem Biophys Res Commun* 281:562-568). Antibody is delivered i.p. (200 ug/0.2 ml/mouse) every other day for 8 days both before (days 14-22) and during disease (24 hours

after onset) as described above. Normal rabbit immunoglobulin and PBS are used as negative controls. Mice immunized with collagen are analyzed macroscopically and histologically as described above.

[0128] **Results.** It is found that administration of FARP protein to mice before disease onset can hasten the onset of disease, and that administration after disease onset can exacerbate disease symptoms and increase vasculature in the inflamed paws. Thus, in preferred embodiments, FARP is deleted by antibody. In alternative embodiments, a FARP knockout in DBA/1 mice is generated. Additionally, since FARP mRNA is synthesized in the rat embryo, it is implicated in embryonic development. In preferred embodiments, the antibody produced as described herein can block or interfere with the function of FARP. A polyclonal antibody produced in rabbits is optimized by using an affinity column made of the recombinant protein to purify the antibody. An alternative approach is to generate a monoclonal antibody. An advantage of using anti-FARP antibodies is the benefit of an antibody as a therapeutic agent.

Example 23

Involvement of FARP in angiogenesis

[0129] FARP mRNA and protein are localized to the vascular endothelium in arthritic paws of CIA mice. Study of protein levels in such mice indicates that FARP protein levels correlate with FARP mRNA levels. Cells expressing FARP mRNA and protein during CIA are identified, and the kinetics of expression of FARP protein during CIA permits design of therapies and testing of candidate drugs having a specific and localized action on FARP mRNA and protein. Preferred therapies and drugs result in enhanced or normalized apoptosis of vascular endothelial cells in the arthritic joint, leading to a diminution or reversal of disease symptoms.

WHAT IS CLAIMED IS:

1. A method for the diagnosis and analysis of autoimmune disease or arthritides, in a patient, comprising:

obtaining a patient sample containing mRNA;

analyzing gene expression using the mRNA that results in a gene expression signature of that mRNA, wherein said gene expression signature comprises the identification and quantitation of gene expression from genes that have been identified as being differentially expressed in RA; and

using that gene expression signature to diagnose or analyze the autoimmune disease or arthritide in said patient, wherein said gene expression of at least about 60% of said genes correlates with that of said gene signature.

2. The method of Claim 1 wherein said autoimmune disease or arthritides are selected from the group consisting of: Rheumatoid Arthritis, Lupus, Ankylosing Spondylitis, fibrositis, fibromyalgia, osteoarthritis, Gout, Juvenile Rheumatoid Arthritis, and an autoimmune disease caused by an infectious agent.

3. The method of Claim 1 wherein said autoimmune disease or arthritide is rheumatoid arthritis.

4. The method of Claim 1 wherein said patient is selected from the group consisting of: a human, a primate, a dog, a cat, a horse, and a sheep.

5. The method of Claim 1, wherein said analysis is selected from the group consisting of: an analysis of severity of the disease, an analysis of pain manifestation, an analysis of deformity, an analysis of treatment methods, and an analysis of treatment efficacy.

6. The method of Claim 1 wherein said gene expression analysis involves at least about 10 genes that are identified as differentially expressed in arthritis.

7. The method of Claim 1 wherein said gene expression analysis involves at least about 50 genes that are identified as differentially expressed in arthritis.

8. The method of Claim 1 wherein said gene expression analysis involves at least about 100 genes that are identified as differentially expressed in arthritis.

9. The method of Claim 1, wherein said genes identified are expressed at least about 1.5 fold higher or lower than normal.

10. The method of Claim 1, wherein said genes identified are expressed at least about 2 fold higher or lower than normal.

11. The method of Claim 1, wherein said genes identified are expressed at least about 3 fold higher or lower than normal.

12. The method of Claim 1, wherein said genes are selected from the group consisting of the 385 genes or ESTs in Table 1 (SEQ ID NOS:1-385), homologs, or variant thereof.

13. The method of Claim 1, wherein said genes are selected from the group consisting of: the genes in cluster A.

14. The method of Claim 13, wherein the genes in cluster A are down-regulated (SEQ ID NOS:1-37) at least about 2 fold.

15. The method of Claim 1, wherein said genes are selected from the group consisting of: the genes in cluster B.

16. The method of Claim 15, wherein the genes in cluster B are up-regulated (SEQ ID NOS:1-37) at least about 2 fold only in late or severe disease.

17. The method of Claim 1, wherein said genes are selected from the group consisting of: the genes in cluster C.

18. The method of Claim 17, wherein the genes in cluster C are up-regulated (SEQ ID NOS:1-37) at least about 2 fold only in early or mild disease.

19. The method of Claim 1, wherein said genes are selected from the group consisting of: the genes in cluster D.

20. The method of Claim 19, wherein the genes in cluster D are up-regulated (SEQ ID NOS:1-37) at least about 2 fold in early or mild disease and more in late or severe disease.

21. The method of Claim 1, wherein said genes are selected from the group consisting of: the genes in cluster E.

22. The method of Claim 21, wherein the genes in cluster E are up-regulated (SEQ ID NOS:1-37) at least about 2 fold in both early or mile and late or severe disease.

23. The method of Claim 1 wherein said differentially expressed genes are the 385 genes identified as SEQ ID NOS:1-385.

24. The method of Claim 1 wherein if the genes in clusters B or D are upregulated, the disease is diagnosed as severe.

25. The method of Claim 1 wherein if the genes in cluster A are upregulated, the disease is diagnosed as moderate to low-grade.

26. The method of Claim 1, wherein said gene expression of at least about 70% of said genes correlates with that of said gene signature.

27. The method of Claim 1, wherein said gene expression of at least about 80% of said genes correlates with that of said gene signature.

28. The method of Claim 1, wherein said gene expression of at least about 90% of said genes correlates with that of said gene signature.

29. The method of Claim 1, wherein said gene expression of at least about 95% of said genes correlates with that of said gene signature.

30. A method for the treatment of RA comprising:
down-regulating at least one of the genes identified in clusters B through D.

31. The method of Claim 30 wherein said down-regulation is by adding antisense oligonucleotides specific for the gene that is being down-regulated.

32. The method of Claim 30 wherein said down-regulation is by adding or expressing an repressor of the gene that is being down-regulated.

33. A method for the treatment of RA comprising:

up-regulating at least one of the genes in cluster A.

34. The method of claim 33 wherein said up-regulation is by adding or expressing a transcriptional activator of the gene that is being up-regulated.

35. The method of claim 33 wherein said up-regulation is by adding a vector that expresses the protein encoded by the gene that is being up-regulated.

36. A method for the identification of genes for targeting in the treatment of rheumatoid arthritis in a mammal other than a mouse, comprising:

identifying homologs of SEQ ID NOS:1-385.

37. A method for the diagnosis of rheumatoid arthritis in a mammal, comprising:

obtaining a tissue or fluid sample from a diseased patient;

isolating mRNA from said sample;

using the isolated mRNA to analyze the gene expression of at least about 40 genes, selected from the group consisting of SEQ ID NOS:1-385 or a homolog thereof, obtaining a fingerprint of the patient's gene expression;

identifying whether at least about 60% of said fingerprint is at least about 2 fold differentially expressed from that of a normal patient.

38. An array or a genechip, specific for rheumatoid arthritis, comprising at least 10 of the genes selected from the group consisting of SEQ ID NOS:1-385 or homologs thereof.

39. The array or genechip of Claim 38, comprising at least 40 of the genes selected from the group consisting of SEQ ID NOS:1-385 or homologs thereof.

40. The array or genechip of Claim 38, comprising at least 50 of the genes selected from the group consisting of SEQ ID NOS:1-385 or homologs thereof.

41. The array or genechip of Claim 38, comprising at least 75 of the genes selected from the group consisting of SEQ ID NOS:1-385 or homologs thereof.

42. The array or genechip of Claim 38, comprising at least 100 of the genes selected from the group consisting of SEQ ID NOS:1-385 or homologs thereof.

43. An array or a genechip, specific for rheumatoid arthritis consisting essentially of, at least 10 of the genes selected from the group consisting of SEQ ID NOS:1-385 or homologs thereof.

44. The array or genechip of Claim 43, consisting essentially of at least 40 of the genes selected from the group consisting of SEQ ID NOS: 1-385.

45. The array or genechip of Claim 43, consisting essentially of SEQ ID NOS:1-385.

46. The array or genechip of Claim 38, wherein said genes allow for the identification of the severity of the disease.

47. The array or genechip of Claim 38, wherein said genes allow for the prognosis of the disease.

48. The array or genechip of Claim 38, wherein said genes allow for the diagnosis of the disease.

49. The array or genechip of Claim 38, wherein said genes allow for the identification of the most efficacious treatment of the disease in a specific patient.

50. A method for the diagnosis or analyses of autoimmune disease or rheumatoid arthritis, comprising

obtaining mRNA from a patient;

using the mRNA as a probe for the analysis of the array or genechip of Claim 38;

comparing the results obtained with those of a normal patient.

51. A method of screening the efficacy of a candidate drug *in vitro* for the treatment of collagen-induced arthritis comprising:

identifying vascular endothelial cells expressing FARP mRNA and protein;

introducing a candidate drug to said endothelial cells; and

evaluating whether said candidate drug causes enhanced or normalized apoptosis of vascular endothelial cells.

52. A method of reducing the symptoms associated with collagen-induced arthritis comprising:

identifying a subject suffering from collagen-induced arthritis; and

administering a compound effective to deplete at least one of the group of FARP mRNA, FARP protein, FARP receptor binding, and FARP activity.

53. The method of claim 52, wherein said compound is an anti-FARP antibody.

54. The methof of claim 53, wherein said antibody interferes with binding of FARP to a FARP receptor.

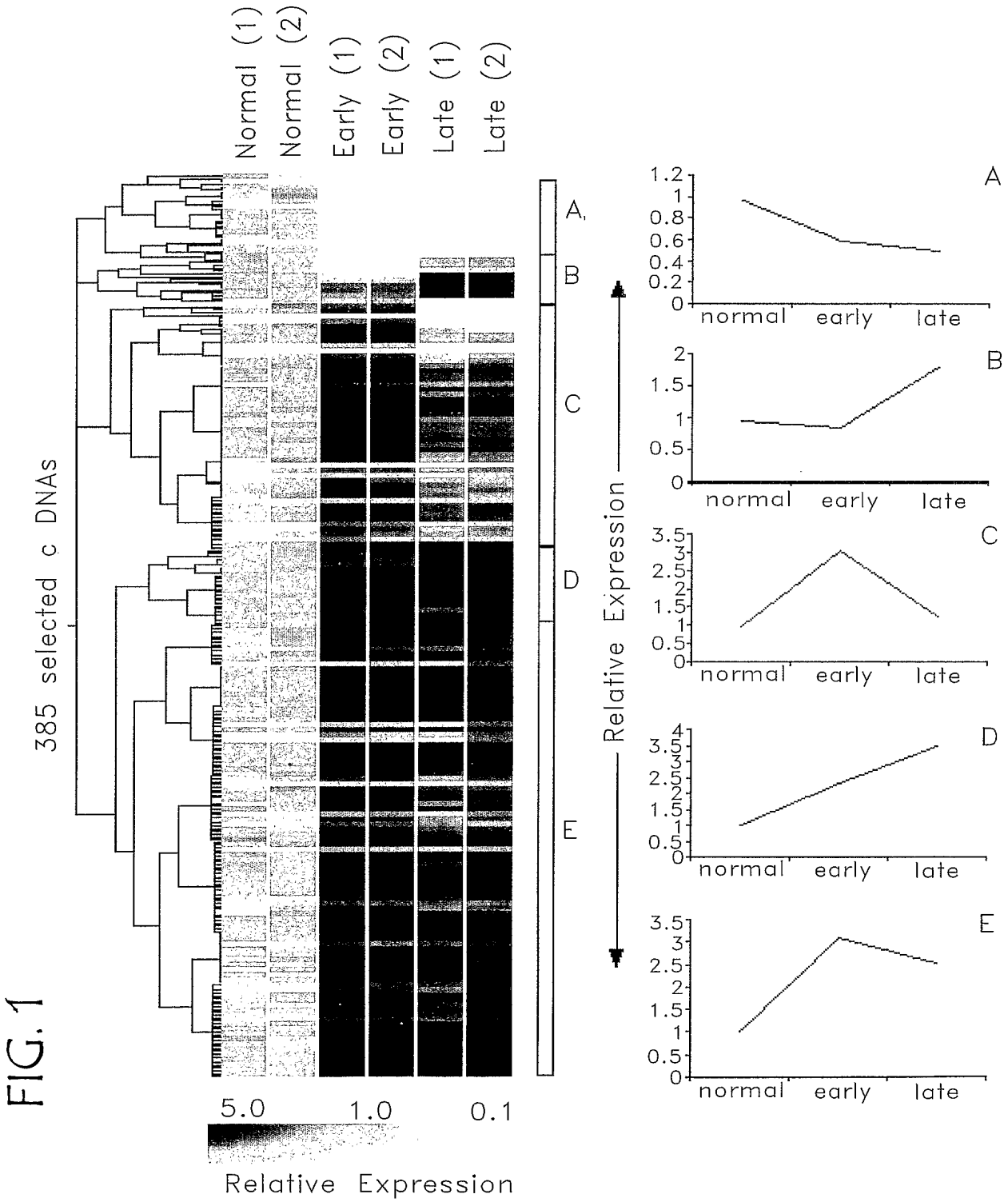

Normal (1)
Normal (2)
Early (1)
Early (2)
Late (1)
Late (2)
385 selected c DNAs
A
B
C
D
E
5.0
1.0
0.1
Relative Expression
Relative Expression
normal
early
late
A
B
C
D
E

FIG. 1

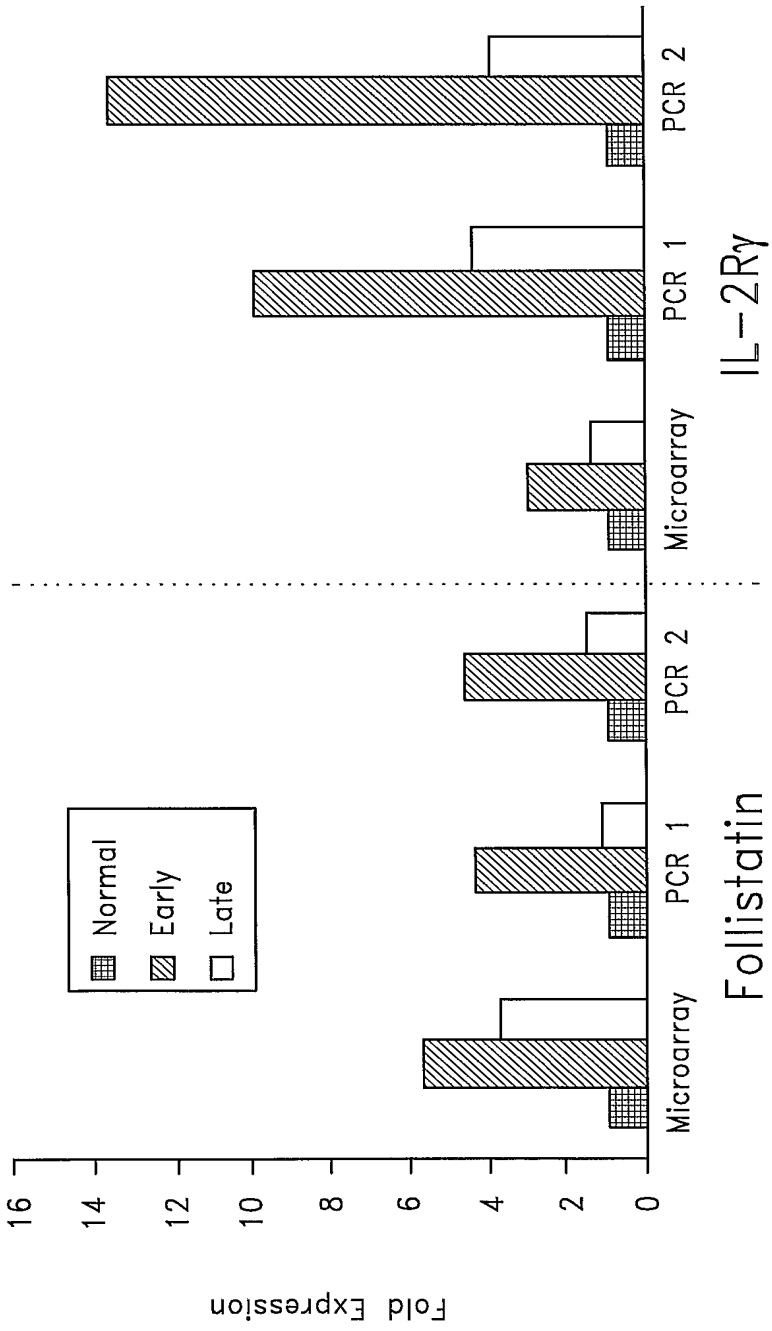
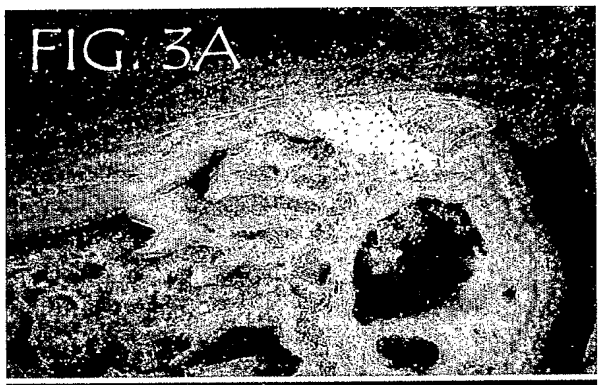
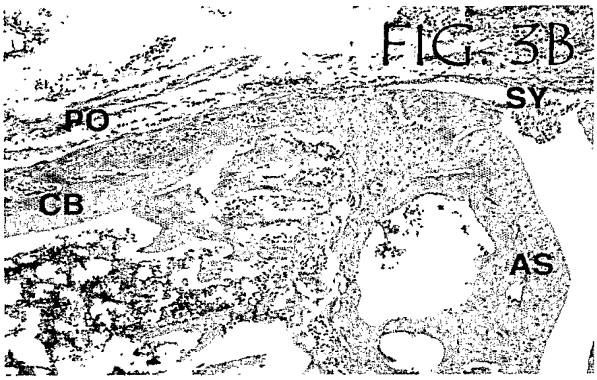
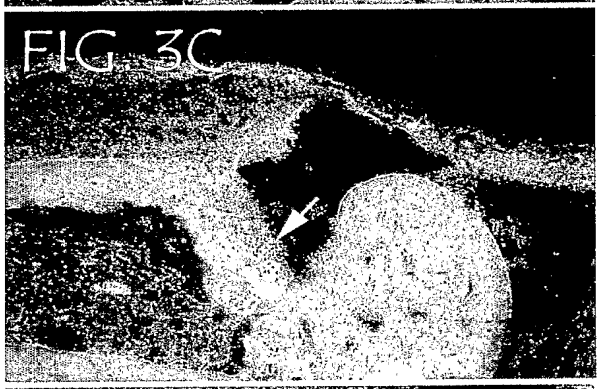

Fold Expression
16
14
12
10
8
6
4
2
0
Normal
Early
Late
Microarray
PCR 1
PCR 2
Follistatin
Microarray
PCR 1
PCR 2
IL-2Rγ

FIG. 2


FIG. 3A


FIG. 3B
SY
PO
CB
AS


FIG. 3C

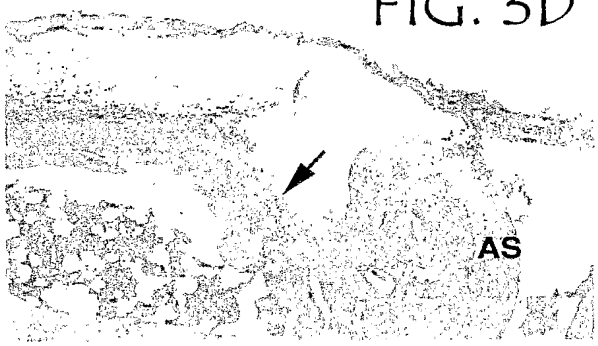
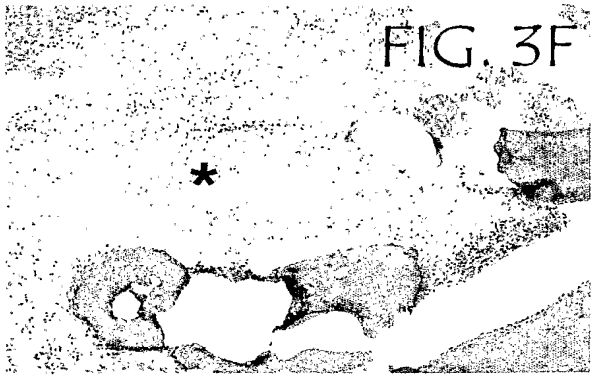

FIG. 3D
AS


FIG. 3E


FIG. 3F

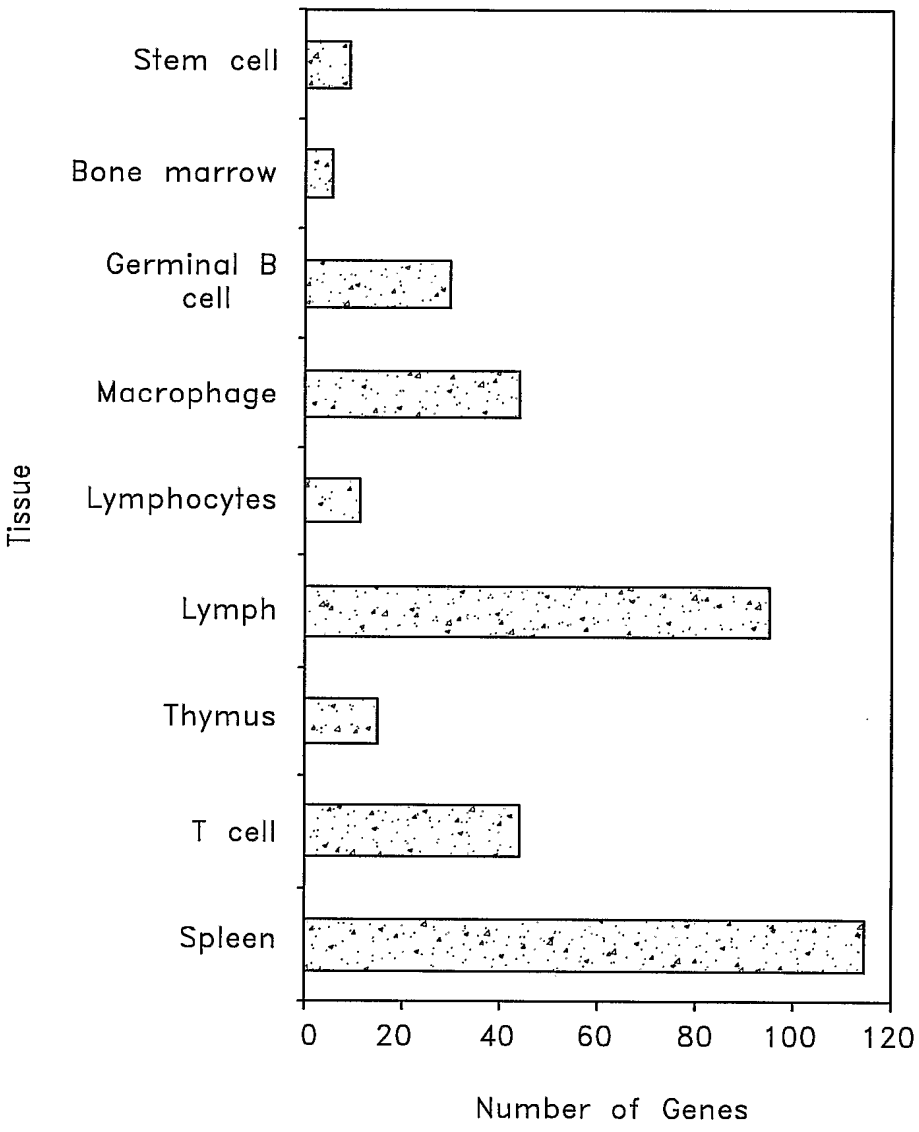

Stem cell
Bone marrow
Germinal B cell
Macrophage
Lymphocytes
Lymph
Thymus
T cell
Spleen
Tissue
0
20
40
60
80
100
120
Number of Genes

FIG. 4

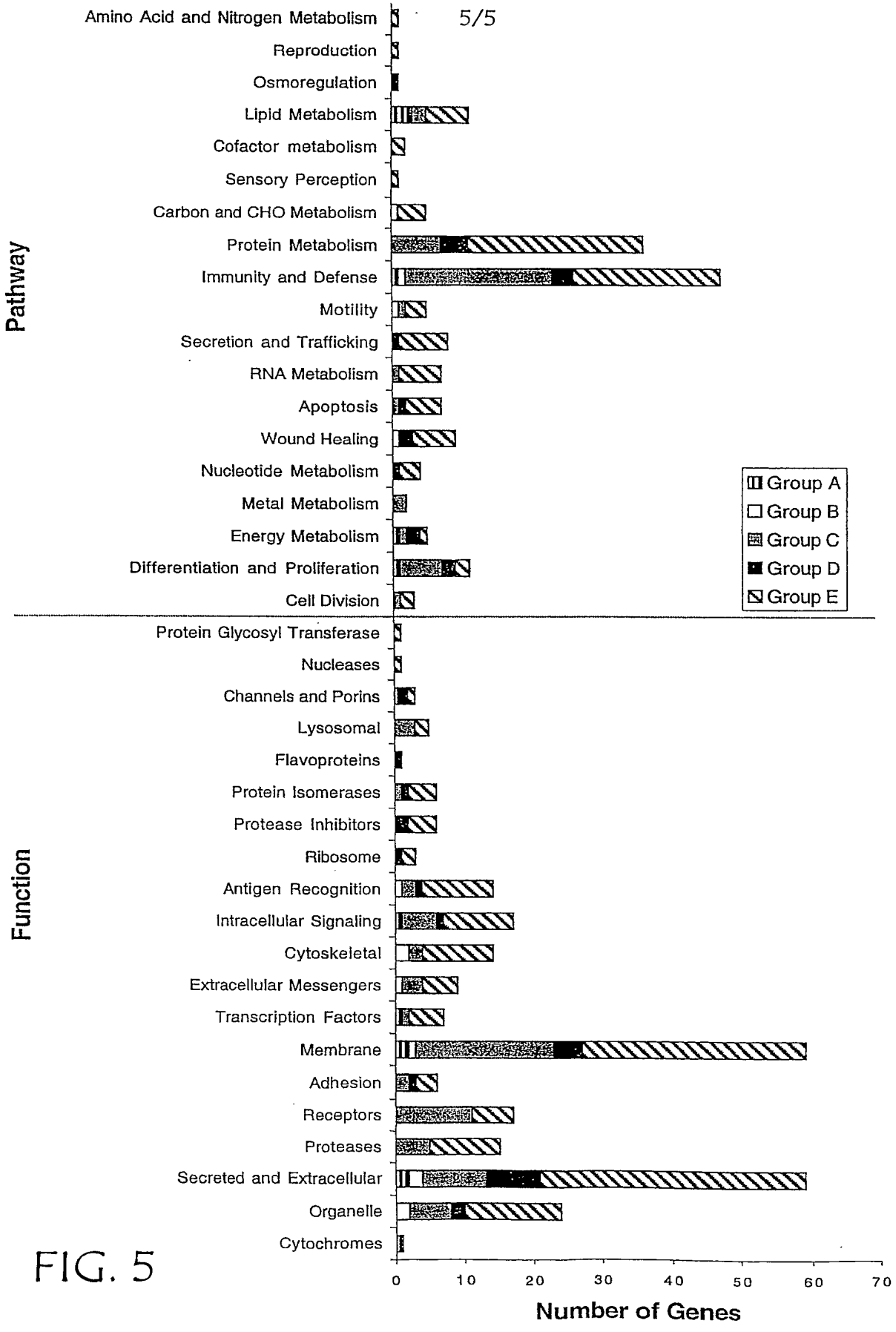

Pathway
Amino Acid and Nitrogen Metabolism
Reproduction
Osmoregulation
Lipid Metabolism
Cofactor metabolism
Sensory Perception
Carbon and CHO Metabolism
Protein Metabolism
Immunity and Defense
Motility
Secretion and Trafficking
RNA Metabolism
Apoptosis
Wound Healing
Nucleotide Metabolism
Metal Metabolism
Energy Metabolism
Differentiation and Proliferation
Cell Division
Group A
Group B
Group C
Group D
Group E
Function
Protein Glycosyl Transferase
Nucleases
Channels and Porins
Lysosomal
Flavoproteins
Protein Isomerases
Protease Inhibitors
Ribosome
Antigen Recognition
Intracellular Signaling
Cytoskeletal
Extracellular Messengers
Transcription Factors
Membrane
Adhesion
Receptors
Proteases
Secreted and Extracellular
Organelle
Cytochromes
0
10
20
30
40
50
60
70
Number of Genes

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/35433

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/68
US CL : 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6,69.1; 702/19,20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,445,940 A (BRENNER et al.) 29 August 1995, see entire document.	1-11, 26-29, and 51-54
Y	US 5,395,753 A (PRAKASH) 07 March 1995, see entire document.	1-11, 26-29, and 51-54
Y	US 6,268,142 B1 (DUFF et al.) 31 July 2001, see entire document.	1-11, 26-29, and 51-54

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
03 May 2003 (03.05.2003)

Date of mailing of the international search report
04 JUN 2003

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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/35433

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 12-25 and 30-50
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Please See Continuation Sheet

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box I Reason 2:

Claims 12-25 and 30-50 were unsearchable because they are directed to specific sequences regarding specific genetic loci which are either in cited clusters or cited via SEQ ID Nos., neither of which have been disclosed in a searchable manner because the sequence listing supplied is unacceptable due to RNA sequences being listed to include thymidine residues given via the nucleotide symbol "t" wherein RNA does not contain such a nucleotide residue bur rather contains "u" as uridine. Therefore the sequence listing could not be processed due to not being entered for searching.

Continuation of B. FIELDS SEARCHED Item 3:

CAS, WEST, BIOSIS, MEDLINE, BIOTECH ABS., WPI, and EMBASE, covering search terms: autoimmune, disorder, arthritis, rheumatoid, mRNA, expression, array, microarray, hybridize, overexpressed, increased, expression, upregulated, and genes

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Published:
— *with international search report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(48) Date of publication of this corrected version:
15 April 2004

(15) Information about Correction:
see PCT Gazette No. 16/2004 of 15 April 2004, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/072827 A1

(54) Title: METHOD FOR DIAGNOSIS AND TREATMENT OF RHEUMATOID ARTHRITIS

(57) Abstract: The onset and progression of chronic autoimmune diseases, including human rheumatoid arthritis (RA) are likely determined by differential expression of genes that influence inflammatory and immune responses. The collagen-induced arthritis (CIA) mouse model for RA exhibits many of the same genetic and immunological features of RA; however, the profiles of gene expression during the inflammatory and immune responses of CIA or RA have not been well characterized. Previous studies have demonstrated that mRNA levels, particularly that of cytokines, can change over the course of CIA. To determine the contribution of various genes in the pathogenesis of CIA, microarray technology was used to simultaneously monitor 8,734 target cDNAs to discover arthritic stage-specific genes. The resulting gene expression profile identified 333 genes that were at least 2-fold up-regulated in all synovial samples: normal, acute disease and chronic disease. In addition, 385 disease-specific genes were identified that were greater than or equal to 2-fold over- or under-expressed in the disease state as compared to normal synovium. Clustering analysis among the arthritic states allowed for the identification of four distinct kinetic expression patterns based on differential expression levels in normal, acute disease and chronic disease synovial samples.